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COMPETITIVENESS OF SLOVENIAN AGRO-FOOD SECTOR

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ABSTRACT
The main objective of this article is to present an overview of the Slovenian food industry economic development in the period before the Slovenian EU accession and the first five years of the EU membership. The accession to the EU was certainly the most comprehensive change of economic environment since the state gained its independence. Nevertheless, already during the pre-accession period the changes intensified restructuring processes and increased pressures to the business performance of the sector. The agro-food industrial complex, however, is among the sectors of the acceding economies for which EU enlargement traditionally brings the most radical changes. Price level and cost differences, hardly comparable production structures, but mostly disparities in types and comprehension of the economic policies required large adjustments and caused notable economic pressures. Based on a framework of economic indicators the paper tries to answer some of the most recurrent questions related to the restructuring process of Slovenian food industry in the last decade. First we present past sectoral business performance using the appropriate mezzo-economic accountancy indicators, whereas in the central part of the paper four key restructuring determinants are analysed and commented. The determinants are formulated into the four relevant questions dealing with cost-price developments, relations in the Slovenian agro-food chain, developments of the international trade and trends in the budgetary transfers to support competitiveness. Results confirm that the economic restructuring which had been hindered in the Slovenian food industry during the last decade has been triggered radically after EU accession. This brought to the termination of favourable economic conditions in the highly protected domestic market and deteriorated radically the business performance, and only those food companies which will restructure proactively will sustain in the EU markets.

Keywords: EU accession, restructuring; Slovenia; food processing industry

INTRODUCTION

Accession to the European Union has triggered substantial changes in the business environment of Slovenian food processing industry which intensified restructuring processes and increased pressures to the business performance of the sector. The agro-food industrial complex is among the sectors for which EU enlargements traditionally brought radical changes in the economic environment. Price level and cost differences, hardly comparable production structures, but mostly disparities in types and comprehension of the agriculture policies required large adjustments and caused notable economic pressures (EL-AGRAA, 1994). The largest European Union (EU) enlargement in May 2004 involved ten countries, most of which just completed their transition to market economy, and another two countries with similar attributes – Bulgaria and Romania –joined the Union in 2007, Croatia in 2013. The enlargement of 2004 differs from the previous ones since the margin in economic prosperity is notably larger compared to any previous enlargement. These disparities bring both distinct challenges and opportunities for the new member states and for the incumbent countries of the Union (ARTIS ET AL., 2006). However, neither the scale of the last EU enlargement nor the combination of the patterns and characteristics of agriculture, food processing, and rural economies are comparable with the past enlargements (MACOURS AND SWINNEN, 1997). Therefore, the evaluation of EU enlargement and its implications for the agro-food sectors has drawn considerable research attention.

There are several studies evaluating in a rather detailed manner the EU accession effects for the sectors of Slovenian agriculture (ERJAVEC ET AL., 1997; BOJNEC AND MÜNCH, 2001). The Slovenian agro-food industry has also been investigated; however focused studies are few (e.g. ERJAVEC AND KUHAR, 2000; OECD, 2001).

MATERIAL AND METHOD

There is no generally accepted theoretical framework economic literature, for sectoral competitiveness; similarly there is no consolidated terminological definition. The concept of competitiveness is clearly multidimensional and therefore it is difficult to deal with theoretically as well as empirically. Some of the leading authors (e.g. TRAIL AND DA SILVA, 1996; LALL, 2001) therefore suggest a composite approach specifically designed according to the focus of analysis.

In a strict sense, there is no specific research methodology applied in this paper, however based on a framework of economic indicators we try to answer some of the most recurrent questions related to development of Slovenian food industry in the last decade. Four questions were put under the research scrutiny: 1) Is food industry facing cost - price squeeze?; 2) Who is creaming in the food chain?; 3) Are the trends in international trade with food surprising?; 4) Has the budgetary support to the sector stimulated competitiveness?.

The paper is structured accordingly to the elaborated four questions. Using data acquired from different official sources a series of economic indicators is calculated to elucidate the determinants of Slovenian food manufacturing sector development. In the first part of the paper the situation in the sector is presented using indicators of business performance, efficiency and profitability whereas in the central part, the article tries to answer the elaborated questions and comment competitiveness determinants.

RESULTS

Economic performance of the agro-food sector

In Slovenia the agro-food sector represented 1.46% of value added in GDP in 2010 and 1.65 % in the total employment (SORS, 2012). According to the value added contribution in total manufacturing food industry is the third largest sector in Slovenia. However, in the last years the importance of food industry is declining in all macroeconomic indicators, since in the year 1998 the food industry contributed about 2.8% of Slovenian GDP and almost 2.5% of total employment. Industrial production of food manufacturing has mainly decreasing after the year 2000. Until the year 2003 the production volume somehow stagnated, but in 2004 volume dropped for more than 10% and then remained stable until 2007. In the year 2011 the food manufacturing was at about 10% below the level of 2004 and beverage sector at about 15% lower. Unfavourable conditions in business performance of Slovenian agro-food sector are discernable also from trends in productivity and value added per employee. In the year 2000 agro-food industry attained about 25% better productivity than average manufacturing and about one fifth higher value added per employee. However, the trends in the following years were constantly negative and in the year 2004 the value added per employee in food industry fell below the average of manufacturing (index for 2005 = 92.2), whereas productivity was still slightly above the manufacturing average (2005 = 101.5). In the following years the indicators fell again, where the productivity was still one tenth higher than on average of the processing sector,

whereas the VA/employee was almost one tenth below the same average. Consequently with worsening of productivity and value added creation, also profitability indicators have dropped, however with oscillating patterns in the last years. Trends of the profitability in the period between 2000 and 2007 measured as return to sales (ROS). In the pre-accession period the ROS was around 3.4%, but after the accession the indicator fell to negative value. Since then the results were constantly and steadily improving in order to reach 4.2% in 2007 which was higher than the average of the processing industry in Slovenia (ROA 2007=3.9%). However, after that, the indicator dropped again considerably, particularly due to the excessive loss in the beverage sector.

The brief overview of some key indicators of business performance above shows rather apprehensive changes in Slovenian agro-food sector. The sector has moved from one of the most prosperous industrial activities in Slovenia during the last decade to the average.

Is food industry facing cost - price squeeze?

Price trend comparison at different levels of measurements reveals general information about the economic environment of an industrial sector. *Figure 1* shows movements of price indices for agricultural inputs, producer prices of food, beverages and tobacco, the producer prices of the food processing industry aggregate and import prices of food in the period 2000-2012. The food retail price index generally fluctuates around the inflation rate if there is no radical structural change in the economy. After the year 2004 food retail prices in Slovenia started to lag behind inflation due to the EU accession. Further the retail prices were falling also in the subsequent period. After that lowest point in 2006 slight strengthening is present until the 2007, when the constant trend of food price increase started. In the last months of the 2011 the food prices were at about 30% higher than in 2005 and more than 50% higher than in 2000.

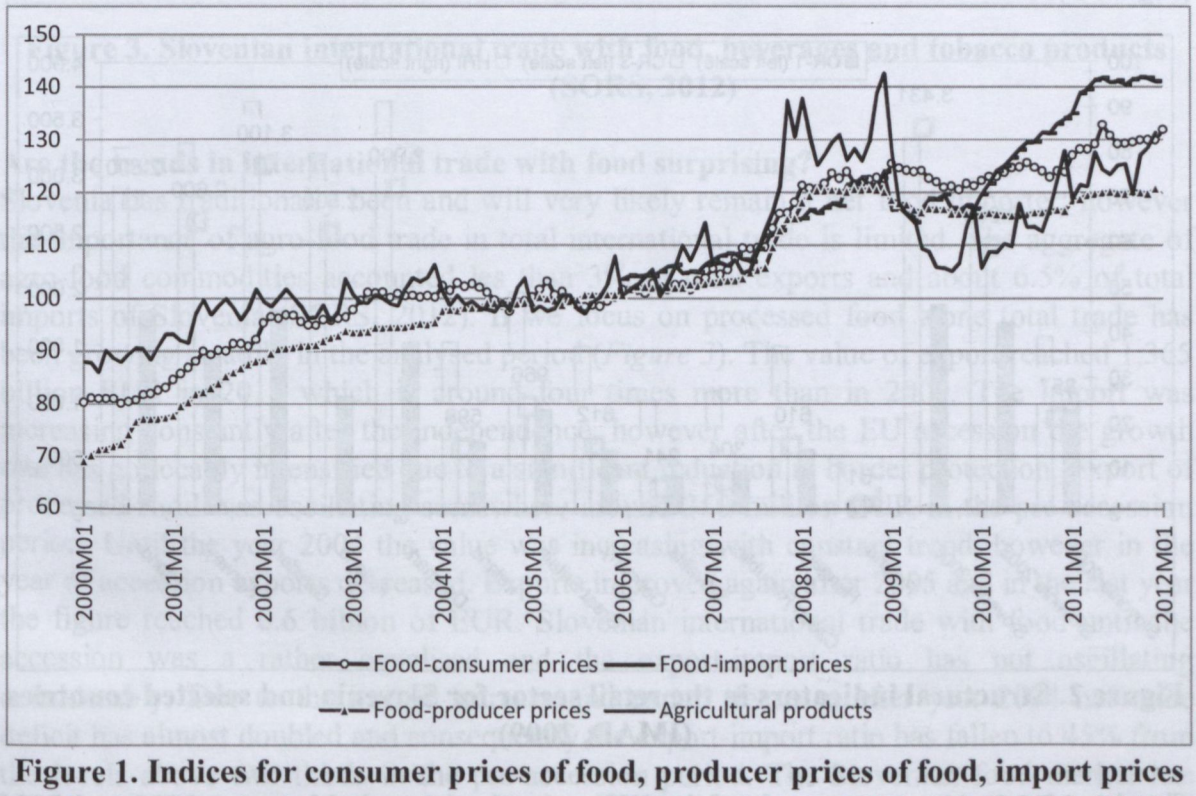

Figure 1. Indices for consumer prices of food, producer prices of food, import prices of food and agricultural products between 2000 and 2012; 2005=100 (SORS, 2012)

In comparison with year 2000 prices of agricultural commodities were lower by 12 % in real terms. In the subsequent years the real price started to increase and then two price

peaks of the decade were reached, one in end of 2007 and the second one in the first years of 2009, bud immediately after the peak the prices started to fall sharply and in the summer 2009 almost reached the average level of 2005. In the following years the prices of agricultural commodities were growing with fluctuating trend, however in the January 2012 the prices were again 30% above the 2005 average. Prior the year 2005 the food producer prices were growing faster than consumer prices and agricultural prices, since they compensate 30% in the first five analysed years. Latter we can observe a significant push-up in the period of agricultural inflation (Y2007), however the price increase was not as high. After that a considerable lagging of the producer prices behind the food consumer prices is evident, which is being explained by the growth of the food import prices. The latter increase by impressive 20 percentage points in the last two years of analysis.
From the price trends and parities revealed above one can not firmly state radical cost-price squeeze for the agro-food manufacturers in Slovenia during the analysed period. Especially the sub-sectors with intensive agriculture input dependence (e.g. dairy, meet, bakery) could benefit form evident improvement of price parity, however this is not confirmed by business performance. A great part of potential positive price-cost development is however absorbed within the retail sector.

Who is creaming in the Slovenian food chain?

Today the food retailers are not only passive intermediates between producers and buyers but a dominant determinant in the agro-food chain. Domination of retail trade in is a global phenomenon (DOBSON ET AL., 2003). Slovenia here is no exemption and it ranges among the European economies with the highest level of concentration in food retailing sector (JUHASZ AND STAUDER, 2005). Already a basic analysis of data reveals significant trends (*Figure 2*).

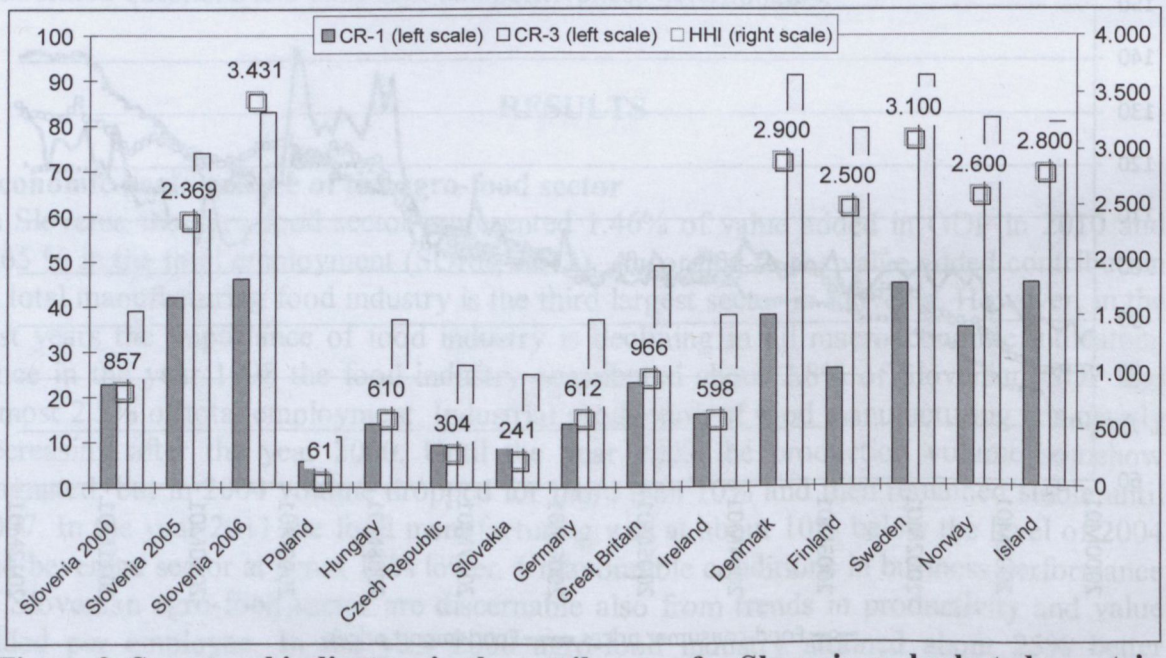

Figure 2. Structural indicators in the retail sector for Slovenia and selected countries (IMAD, 2009)

The data show enormous growth of the HHI value in the period between 2000 and 2006 since the index quadruplicated. In the last year the HHI reached the value of 3.431, a considerably higher value than estimated for Sweden and Denmark which have the amongst the highest HHI in Europe. Also according to the other indicator of the market

structure – namely the Concentration ratio (CR); Slovenian retailing sector is highly saturated. In 2006 the largest Slovenian retailer contributed 46% of the total sectoral revenue, whereas the first three retailers contributed as much as 83%. Both figures are among the highest in Europe. Simultaneously with the retail sector concentration growth also the nature of the supply chain relations has changed considerably. Retailers are imposing many other contractual and operational practices that negatively influence suppliers. Unjustified costs are carried onto agro-food producers which deteriorate their business performance and are of benefit to retailers.

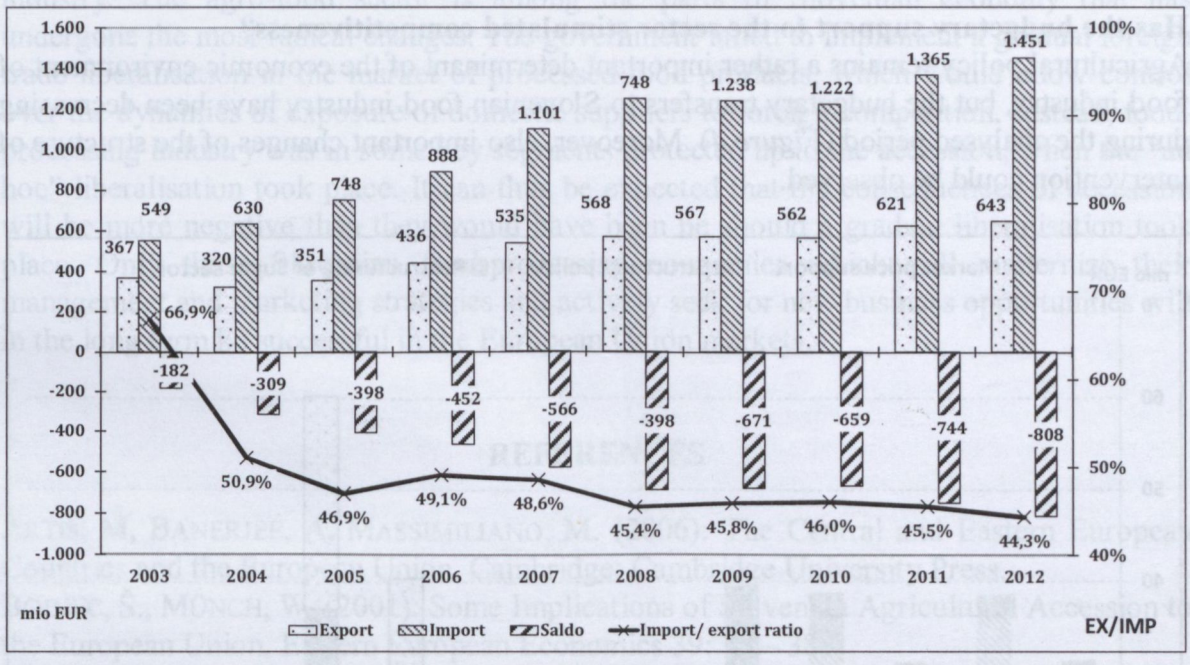

Figure 3. Slovenian international trade with food, beverages and tobacco products (SORS, 2012)

Are the trends in international trade with food surprising?

Slovenia has traditionally been and will very likely remain a net food importer; however the importance of agro-food trade in total international trade is limited. The aggregate of agro-food commodities accounted les than 3% of total exports and about 6.5% of total imports of Slovenia (SORS, 2012). If we focus on processed food alone total trade has been growing steadily in the analysed period (*Figure 3*). The value of export reached 1,365 billion EUR in 2011 which is around four times more than in 2000. The import was increasing constantly after the independence, however after the EU accession the growth rate has noticeably intensified due to a significant reduction in border protection. Export of processed food was oscillating somewhere around 350 million EUR in the pre-accession period. Until the year 2004 the value was increasing with constant trend, however in the year of accession exports decreased. Exports improved again after 2005 and in the last year the figure reached 0.6 billion of EUR. Slovenian international trade with food until the accession was a rather equalised and the export-import ratio has not oscillating considerably. Due to the import increase and export decrease after year 2004 net trade deficit has almost doubled and consequently the export-import ratio has fallen to 45% from the levels above two thirds in the pre-accession period. The Slovenian food market has been highly protected until the EU accession, since for the majority of food groups the effective tariff rate in the pre-accession period has been fluctuating around 10%. From the evaluated import protection one could anticipate the effect of the inclusion to the internal market of the European Union on Slovenian food trade. The effect of "trade creation"

appeared, which is described as a basic effect of trade union (EL AGRAA, 1994).
Also on the export side of the Slovenian trade balance the EU accession effects might be expected from the regional structure of trade, since not less than 60% of the food exports was realised on the markets of Former Yugoslavia. With these countries Slovenia signed preferential trade agreements which made food products competitive in comparisons with other imported products. However, after the accession the trade agreements were abolished. This has immediately resulted in export decrease, but the export switched to the EU markets. In the year 2011 the share reached almost two thirds of the total exports.

Has the budgetary support to the sector stimulated competitiveness?
Agricultural policy remains a rather important determinant of the economic environment of food industry, but the budgetary transfers to Slovenian food industry have been decreasing during the analysed period (*Figure 4*). Moreover, also important changes of the structure of intervention could be observed.

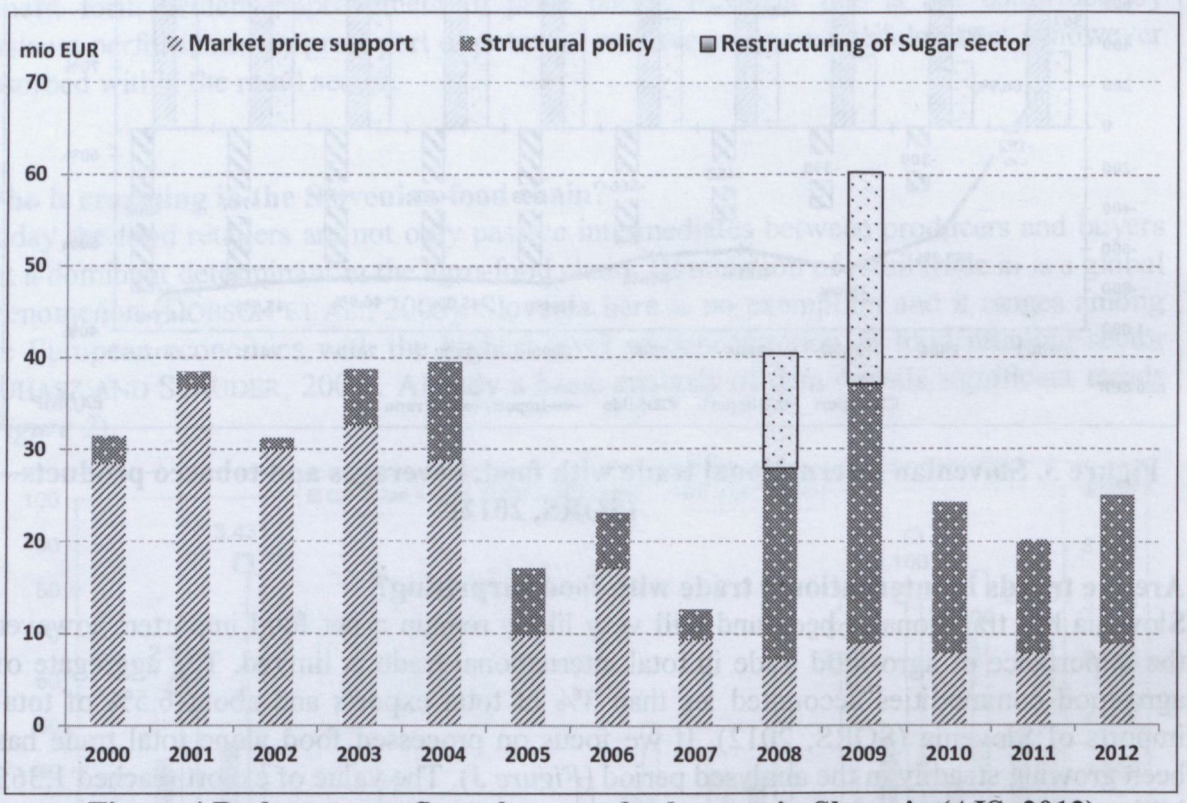

Figure 4.Budgetary outflows for agro-food sector in Slovenia (AIS, 2012)

The total budgetary transfers are divided into Market interventions and Structural policy measures. The first part includes mainly transfers to exporting food enterprises (export subsidy) and the Proportion of this type of support in total transfers was around 90% until 2003, when the share of structural measures increased, but also the value of export subsidies decreased considerably. The export subsidies in combination with bilateral trade agreements had clear distortive implications on competitiveness of Slovenian food sector and importantly influenced the structure of exports. Despite the considerable amount of budgetary transfers to Slovenian food sector, majority of them has not been intended for long term competitiveness improvement, but rather to stabilise domestic agricultural markets and to correct price distortions. Only in the last period the share of structural measures increased, however processes to build sustainable economic competitiveness are demanding and long lasting.

CONCLUSIONS

The economic restructuring that had been hindered in the Slovenian food industry during the last decade has been triggered radically after EU accession which brought to the termination of favourable economic conditions in the highly protected domestic market. Competition from the internal market, but more significantly the reduction of export competitiveness due to the abolition of free trade agreements has caused an important long term deterioration of economic performance in almost all sub-sectors of the Slovenian food industry. The agro-food sector is among the parts of Slovenian economy that has undergone the most radical changes. The government failed to implement a gradual foreign trade liberalisation in the market of processed-food products, which would allow control over the dynamics of exposure of domestic suppliers to foreign competition. Instead, food-processing industry was in some key segments protected up to the accession, when the "ad hoc" liberalisation took place. It can thus be expected that the consequences of accession will be more negative than they would have been be should a gradual liberalisation took place. Only those Slovenian food-processing companies which will modernize their management and marketing strategies and actively seek for new business opportunities will in the long term be successful in the European Union markets.

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RESULTS OF HUNGARIAN WOODCOCK MONITORING

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ABSTRACT

Hunting woodcock in spring is a centuries-old tradition in Hungary. However the EU Birds Directive (79/409/EEC) prohibits hunting during the migration to breeding areas. In order to regularly derogate the EU Birds Directive it was essential to start and maintain a country-wide monitoring system, and to estimate the size and the mortality of woodcock population migrating across Hungary. As there were no similar studies earlier we had a) to develop and test the workability of a long term monitoring programme of Woodcock migration in Hungary in spring and in autumn; b) to describe the dynamics of the migration; c) to detect and evaluate differences among years; d) to estimate the size of migrating population in spring and in autumn; e) to calculate the mortality as the difference of autumn and spring population.

The monitoring programme started successfully, and it is running on a national scale for five years now. We have chosen synchronous observation of flying birds from fixed points during the whole migration period. The observations were performed by local hunters weekly, they observers recorded data on standardized forms. We calculated the mean densities of contacts (woodcocks seen/hectare/hour) for each observation date in each county. Their distribution represents the temporal dynamics and intensity of migration. We estimated the migrating population size in two different ways. First, the densities at the peaks of migration were used for the estimation of a minimal population size. Second, the total population size was estimated using the densities calculated in the whole season. In both cases, the estimation relied on the densities multiplied by the total size of the forested areas in the country.

We detected high variability of contacts in space and time, which fits to the former experience of woodcock hunters. It reflects the highly flexible migratory behavior of woodcocks. Observations in autumn can provide information about migration, but the simple comparison with spring data is problematic because of the behavioral differences. We were constrained to use literature data for the calculation of minimum and total number. According to our results, the hunting bag in Spring in Hungary may be far under the 1% limit that was determined in the Guidance document of Birds Directive. We suppose that such a quantity does not threat the woodcock population.

Keywords: Woodcock, migration, monitoring, game management unit (GMU)

INTRODUCTION

Hunting woodcock (*Scolopax rusticola*) in spring is a centuries-old tradition in Hungary. The annual hunting bag in the last decade (Csányi et. al, 2009) was always less than 10.000 individuals. However the EU Birds Directive (79/409/EEC) prohibits hunting during the migration to breeding areas. An autumn hunting season seems to be a legal solution, but in the Hungarian context, it also could cause more difficulties than it would solve and its influence on the population dynamics is not clear. The Directive allows derogations under controlled conditions and only for a small number of birds [1% of total mortality (natural + hunting) at maximum]. Basic population parameters: size and mortality is needed to estimate the 'small number'. Although there are data about the size of the population – by the official data of Birdlife International it is 10.000.000-26.000.000 individuals globally – these may be very inaccurate and based on experts guesses in most

cases. Only a few countries (France, Russia, Belarus, UK and Portugal) run regular monitoring programs (FOKIN ET AL., 2004; SANDAKOV, 2004; FERRAND ET AL., 2008; MACHADO ET AL., 2008). Moreover, the size of the breeding or wintering population is estimated usually, but insufficient data are available during migration. Several migration routes are known among the wintering areas in South-West Europe and Mediterranean region and breeding areas from Scandinavia to Ural Mountains (FARAGÓ, 2008), but the distribution of migrating woodcocks among different flyways are not well known. In order to regularly derogate the EU Birds Directive was essential to start and maintain a country-wide monitoring system to estimate the size and the mortality of woodcock population migrating across Hungary.

As there were no similar studies earlier we had a) to develop and test the workability of a long term monitoring programme of Woodcock migration in Hungary in spring and in autumn; b) to describe the dynamics of the migration; c) to detect and evaluate differences among years; d) to estimate the size of migrating population in spring and in autumn; e) to calculate the mortality as the difference of autumn and spring population.

MATERIAL AND METHOD

A long-term monitoring programme was initiated by the former Ministry of Agriculture and Rural Development and the Hungarian National Chamber of Hunters (HCH). The programme started in 2009. Data collection and processing have been designed and carried out by Szent István University, Institute for Wildlife Conservation (IWC) which also assumed to evaluate the results.

The area covered by monitoring and the minimal desired number of observation points were determined using annual hunting bag data of 1997-2007. The game management units (GMU), as individual samples, were classified into three categories: permanent (80-100%), occasional (10-70%) occurrence and no occurrence. We calculated the minimum number of samples by rarefaction analysis at 5%, 10% and 15% confidence level. The highest calculated number of samples was 425 at the 5% confidence for the 1st and 2nd zone together.

Table 1. Duration, number of GMUs, monitoring sites and forms of the woodcock monitoring in Hungary 2009-2013 (2013 autumn data are under processing)

Spring

Year	**2009**	**2010**	**2011**	**2012**	**2013**
Duration (weeks)	10	12	12	12	12
Game management units	435	445	448	452	439
Monitoring sites	856	922	922	944	907
Forms	7140	9112	10066	10319	10013

Autumn

Year	**2009**	**2010**	**2011**	**2012**	**2013**
Duration (weeks)	12	14	12	12	-
Game management units	388	422	443	436	-
Monitoring sites	756	846	906	893	-
Forms	7755	10364	10093	9913	-

More GMUs volunteered the monitoring programme, than it was expected (*Table 1*). As majority of GMUs undertook to collect data more than one observation points, the real number of monitoring sites were more than two times bigger than the requirement for the best confidence. The majority of observation points covered forested areas (*Figure 1*).
A network of specialists was organized for the administration of data. It included county coordinators (employees of the Hungarian Chamber of Hunters), representatives of GMUs and observers (participating hunters). GMU representatives collected the observation forms and sent those to the county coordinators each week. County coordinators uploaded the observation data each week to a web server created and maintained by IWC.
We have chosen synchronous observation of flying birds from fixed points during the whole migration period. The base of the monitoring programme was roding survey (FERRAND, 1993). The observations were performed by local hunters weekly (on every Saturday from the end of February to the first week of May in spring, and on every Tuesday from mid-September to early December in autumn). The observers recorded data on standardized forms. Data were: number of contacts (woodcocks seen and/or heard), estimated size of the visible area, duration of the survey, weather conditions and habitat types surrounding the observation point. These data give us snapshots about the different states of the migration. With the comparison of consecutive snapshots we can estimate the dynamics, speed and extent of the migration.

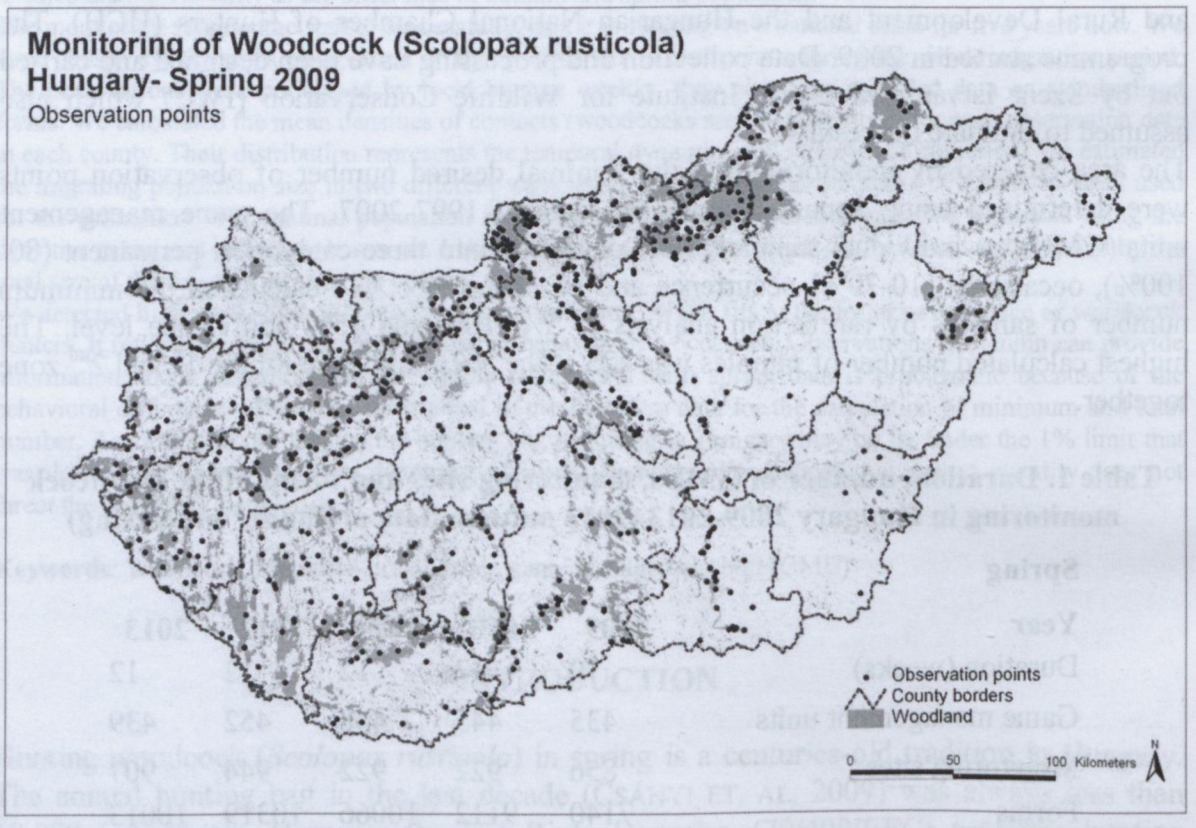

Figure 1. Spatial distribution of the observation points

We calculated the mean densities of contacts (woodcocks seen/hectare/hour) for each observation date in each county. Their distribution represents the temporal dynamics and intensity of migration. We used Kruskal-Wallis test with Dunn's multiple comparisons test to detect differences among the number of contacts reported at the annual peaks of roding intensity.

We estimated the migrating population size in two different ways. First, the densities at the peaks of migration were used for the estimation of a minimal population size. Second, the total population size was estimated using the densities calculated in the whole season. In both cases, the estimation relied on the densities multiplied by the total size of the forested areas in the country.

Data management and statistical analyses were performed using Microsoft Excel 2003; R (v2.15.0) and GrahPad InStat (v3.05)

RESULTS

The observation data were highly variable not only in time (annual, seasonal and weekly) but also geographically. In Spring, the temporal progression of the number of contacts was unimodal in every year (*Figure 2*). We have found difference among the annual peaks (Kruskal-Wallis Statistic KW = 339.95 P <0.0001) (*Figure 2*). The number of contacts in 2013 differed from the data of 2009, 2011 and 2012, but no difference was found compared to the data of spring 2010.

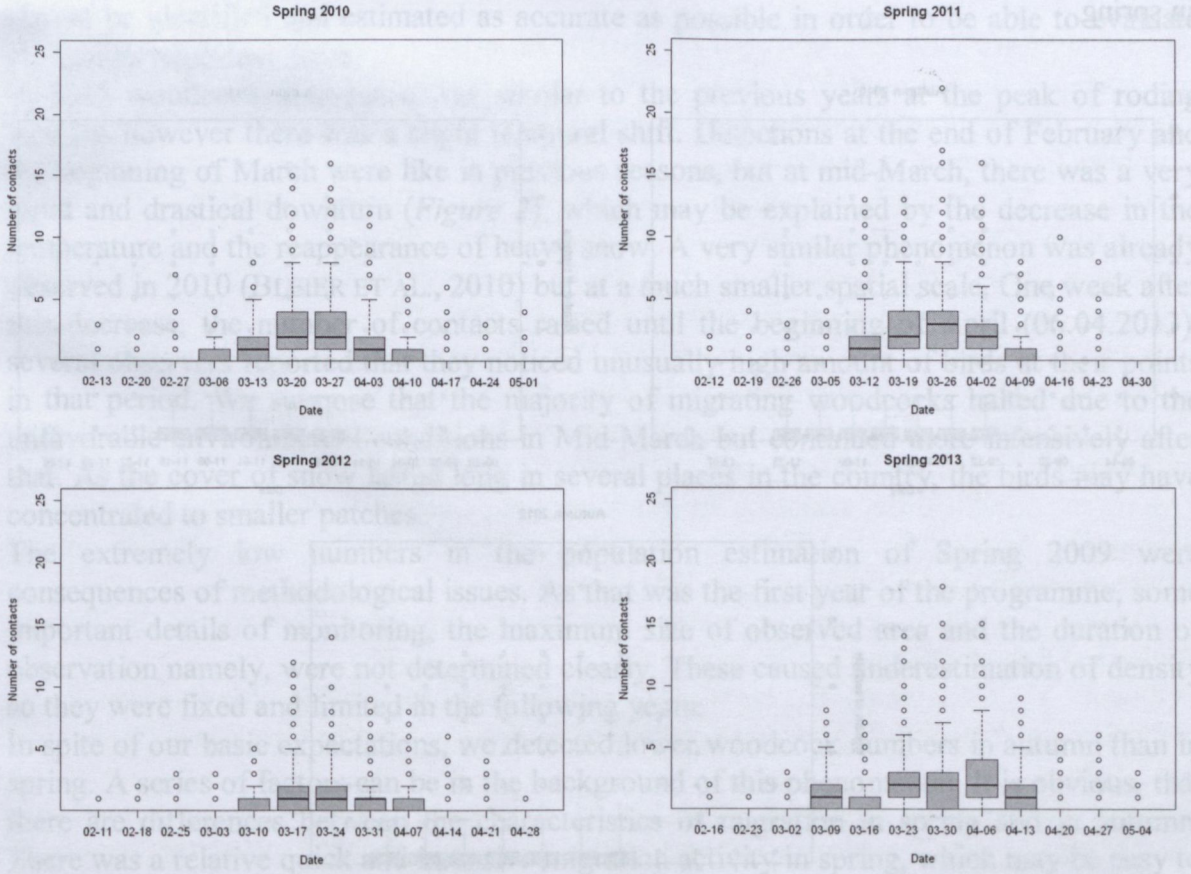

Figure 2. The woodcock detection dynamics in Hungary in spring 2010-2013

The number of contacts reported in Spring 2012 were the lowest, two or even three times lower than in the previous years at the peak of roding intensity. There was also a notable decrease in the rate of sites where at least one woodcock was detected at the peak that year. Whilst it reached even 90% in the previous years (90.93% in 2009, 88.61% in 2010, 89.98% in 2011) it was only 73% that year. Moreover the rate of sites with at least five detections at the peak was also the lowest so far (19.13% in 2009, 14.81% in 2010, 17.26% in 2011 8.71% in 2012).

The migration dynamics showed less obvious peak in autumn, than in spring (*Figure 3*). The migration seems to be long-drawn-out and more balanced at that time. The numbers of contacts were lower in Autumn than in Spring in each year (*Figure 3*). Consequently, mortality cannot be estimated by a simple comparison of Spring and Autumn values. We were constrained to use literature data for the calculation of minimum and total number. According to the Guidance document on hunting under Council Directive 79/409/EEC on the conservation of wild birds (HTTP1) the mortality of the young birds (<1 year old) varied between 54-72%, and 39-54% by adult woodcocks. So, we used 50% as general mortality ratio.

The estimated minimum population size varied between 4 174 929 (2012) and 6 890 809 (2010) individuals, except in 2009 when it was 1 483 224 only. The estimated total number of migrating woodcocks was the lowest in 2012 with 15 210 835 individuals and the highest in 2013 with 28 317 756 individuals. The 2009 data was also extremely low 5 924 688 individuals.

The rate of the hunting bag in Hungary (CSÁNYI ET AL., 2011; CSÁNYI ET AL., 2012; CSÁNYI ET AL. 2013) compared to the annual mortality rate estimations (birds shot/1% of estimated mortality)varied between 0.36% and 0.52% calculated from numbers of the annual peaks of migration and 0.11% up to 0.14% concerning the whole migration period in spring.

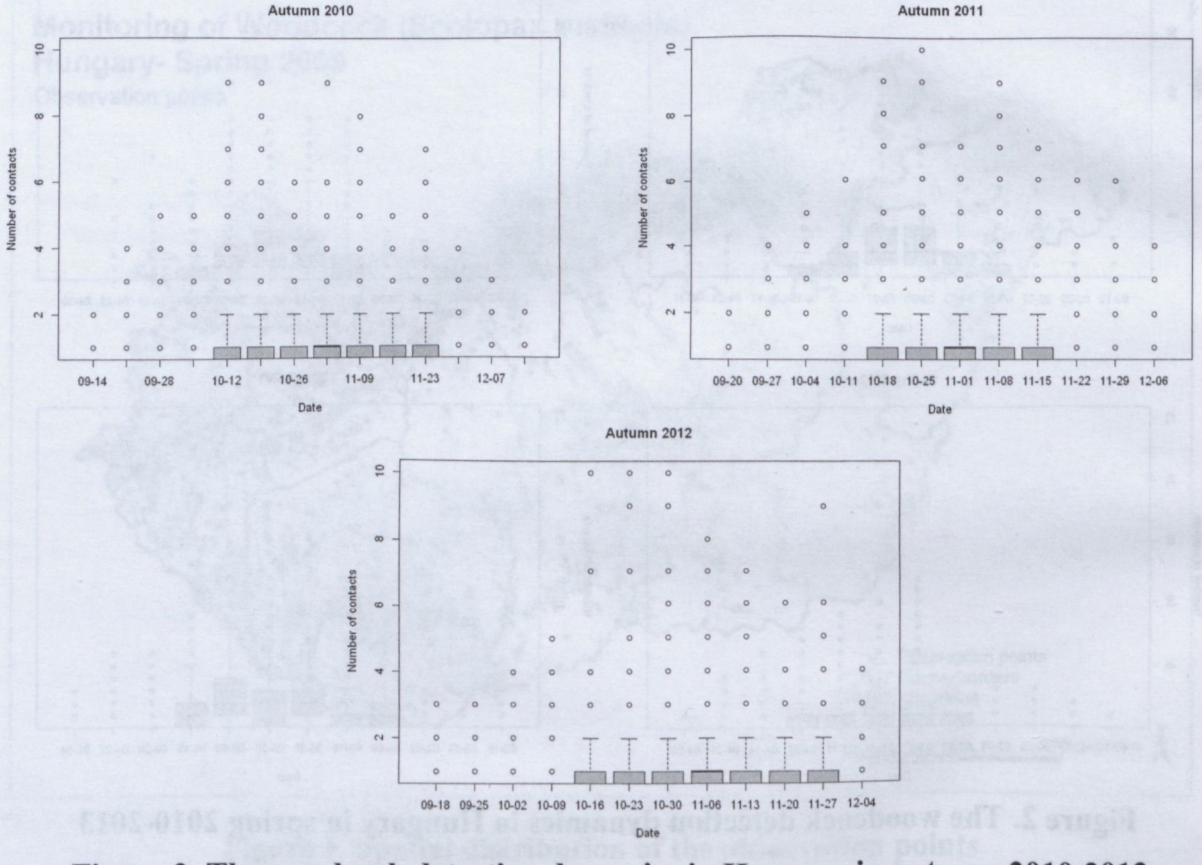

Figure 3. The woodcock detection dynamics in Hungary in autumn 2010-2012

DISCUSSION AND CONCLUSIONS

The monitoring programme started successfully, and it is running on a national scale for five years now. Testing the workability, gathering methodology experiences and further development were the most important goals in its first period. It is clear now that the

Hungarian hunters are able to cooperate with each other and to solve a task of such a magnitude. Our aim is to continue and improve monitoring of the species in the future based on the knowledge gathered along that period.

We detected high variability of contacts in space and time, which fits to the former experience of woodcock hunters. It reflects the highly flexible migratory behavior of woodcocks. Migration can be affected by different abiotic factors like temperature, wind and snow cover.

The peak of spring 2012 was the lowest in the last four years. As the numbers of contacts in 2013 were similar to the ones of the previous years, the low values reported in 2012 (SCHALLY ET AL., 2012) might not indicate a negative trend in the population. According to our results we conclude that the decrease we noticed in the number of contacts in 2012 could be caused by a temporary, significant decrease in the size of suitable areas for woodcock. That year's spring and even the winter of 2011 was extremely dry which is known to be unfavorable for earthworm feeders such as the Eurasian woodcock. Due to such conditions the birds could have decided to avoid or escape these dry areas along their migration. It is not clear yet whether it was an extreme case or how often can it occur in the future. However it draws our attention on factors which can affect the migration of woodcocks dramatically and yet we can hardly influence. The effects of these factors should be identified and estimated as accurate as possible in order to be able to evaluate our results regarding them.

In 2013 woodcock abundance was similar to the previous years at the peak of roding activity, however there was a slight temporal shift. Detections at the end of February and the beginning of March were like in previous seasons, but at mid-March, there was a very rapid and drastical downturn (*Figure 2*), which may be explained by the decrease in the temperature and the reappearance of heavy snow. A very similar phenomenon was already observed in 2010 (BLEIER ET AL., 2010) but at a much smaller spatial scale. One week after this decrease, the number of contacts raised until the beginning of April (06.04.2013), several observers reported that they noticed unusually high amount of birds at their points in that period. We suppose that the majority of migrating woodcocks halted due to the unfavorable environmental conditions in Mid-March but continued more intensively after that. As the cover of snow lasted long in several places in the country, the birds may have concentrated to smaller patches.

The extremely low numbers in the population estimation of Spring 2009 were consequences of methodological issues. As that was the first year of the programme, some important details of monitoring, the maximum size of observed area and the duration of observation namely, were not determined clearly. These caused underestimation of density so they were fixed and limited in the following years.

In spite of our basic expectations, we detected lower woodcock numbers in autumn than in spring. A series of factors can be in the background of this phenomenon. It is obvious, that there are differences between the characteristics of migration in spring and in autumn. There was a relative quick and intensive migration activity in spring, which may be easy to explain from a biologist's point of view. The birds that reach the breeding areas faster can occupy sites of a better quality. They can be more successful, they may have more time to raise their broods and the young ones can start the migration to the wintering areas in a better condition. Migration in autumn lasted relatively longer, and birds probably arrived in Hungary in several smaller waves. It is also possible that some of them stay in the Carpathian basin for winter.

The detectability of woodcock in autumn is significantly lower than in spring. In spring, woodcocks can be detected by sight and listening but only by sight in autumn. The lower detectability can cause biased population size estimation. Observations in autumn can

provide information about migration, but the simple comparison with spring data is problematic because of the behavioral differences.

Finally we conclude that the hunting bag in Spring in Hungary may be far under the 1% limit that was determined in the Guidance document of Birds Directive. We suppose that such a quantity does not threat the woodcock population.

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We are grateful to everyone who persists in collecting data of woodcock from the beginning of the programme. We are also thankful to the Hungarian National Chamber of Hunters and the Ministry of Rural Development for their help in coordination. The research was also supported by the Research Faculty Grant of the Hungarian Ministry of Human Resources (7629-24/2013/TUDPOL).

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HTTP1:

http://ec.europa.eu/environment/nature/conservation/wildbirds/hunting/guide_en.htm

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PACKING INNOVATION FOR FOOD – ATTRACTIVE FOR EYES, EASY TO CARRY AND ENVIRONMENT FRIENDLY

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ABSTRACT

One purpose of cognitive ergonomics is to present products in such an innovative way that the customer brain is influenced during the shopping in supermarkets and retail.

Packing innovations can increase a longer shelf life for fruits, vegetables and at home use. The design of the packing however makes the product virtually enjoyable. Our potential consumers can be educated from start of their life to love brands for a life time.

Some designs may make known food products friendly, sensual and bond with personal connection. New laws in EU for lifting in retail demand appropriate shelves solutions and EU laws for environmentally friendly products force industry to develop new products that will reduce the use of plastic shopping bags.

This paper discussed the use of a new packing material and technique as a packing solution for some products like eggs and champagne.

Keywords: ergonomics, retail, innovative packing designs

INTRODUCTION

Reason to study ergonomics for agriculture, food products, retail and international food trade is in fact to design innovative packing solutions that are made in accordance with guidelines often easer for use and has physical capabilities for young and elderly population. Ergonomics has become an important pillar for product development and industrial design engineering in particular (DIRKEN, 1997). Product ergonomics is young discipline connected with industrial innovations and multiplies science disciplines. Ergonomics products is or not in connection with effectiveness and efficiency, safety and more comfortable for use by the targeted group of users. Effectiveness is measured by how much one product can be in use and if less than 100%, the product is less effective. Comfort is measured with discomfort of use. Safety and security of the product speak by itself. Efficiency might be measured for example with time: how much time is needed for opening or similar till moment of use. Elementary factors by product ergonomics are: people, product, interaction, and surrounding (space). One product can have several meanings and functionality, the physical use, help middle, informative, prototype, for one way or multiply use, optimal (DAAMS, 2011).

There are three kinds of ergonomics:

1) **Sensory**-connected with human sense for to see, listen and taste;
2) **Cognitive** – is in connection of understanding, decision and use;
3) **Physical** –is in connection with a body shape, movement, power and resistance.

Daams points that product ergonomics combine disciplines like work; consumer's and design developing ergonomics to produce products for large group of people. Technical with functionality use defined modern product in categories. The first ergonomics products date from *Paleolitic* Era (in old Greeks: *Palaios*-old and *Lithos*-stone) "Old Stone age", when first human – *Homo erectus* and *Homo habilis* began to use first stone tools for their living and to survive (GUISEPI, 2000). From that time till today, many natural materials

have been used to catch and store food, for transport, packing. Use of two stones to make fire; enabled humans to reduce illness by cooking and frying. People started to copy each other and make new standards for use of food. Industrial revolution made possible to put researcher, designer, producer and user in strong connection. In the half of 20th century tree disciplines try to cover ergonomics:

1) **Psychology**,
2) **Work psychology**,
3) **Technical science**.

The establishment of ergonomics society became after Second World War: Ergonomics Research Society and Institute of Ergonomics and Human Factors (ENGLAND, 1947), Human Factors and Ergonomics Society (USA, 1957), International Ergonomics Association (IEA, 1961), Dutch Association for Ergonomics (De Nederlandse Vereninging voor Ergonomy, 1962) and the worldwide organization IEA (www.iea.cc). Ergonomics contribute to design and evolution of tasks, jobs, products, environments and systems in order to make them compatible with the needs, abilities and limitations of people (DAAMS, 2011). Ergonomics is about designing for people wherever they interact with products, systems or processes (www.ergonomics.org.uk).

MATERIAL AND METHOD

In packing, new technologies can lead to innovative applications which offer valuable improvements compared to existing packing. One recent innovation concerns the invention of a new bio-based material which is made from industrial starch (potato), natural fibres, water and "pre mix". Products are made of this material via a patented injection moulding process and can be composed. The mixed foam is pressed in the machine under high temperature to obtain the desired packing form. This contemplates the performance bio-based material in two different packing applications: egg packing and a champagne or wine bottle packing.

RESULTS

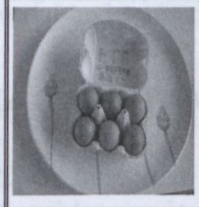
Eggs packing

Eggs belong to the products in the supermarkets customers favour in their shopping basket. This equally appears for the milk, bear, or brad. Worldwide sales of packing design for individuals or "take away" products for single use, is only possible if the popularity of such a product increases. On the other hand, packing these products in packaging which appeals more to the customers than to the present packaging.

Eggs are traditionally typically packed in a tray made from either carton or plastic and available in different types to hold six, twelve, fifteen and eighteen eggs. Requirements to egg packaging are that the breakage rate is minimised in transport and sufficient sturdy construction to absorb shocks. Functionality is second priority. With eggs, which are part of daily food, packing must be surprisingly simple to use. What is the usual perception of consumer when buying dozen eggs? Can the buyer be tempted to make another decision then usual? Eggs are very sensitive to pricing and most of the shoppers will make rational decisions often based on the lowest price. Until now, other factors like appearance and usability of egg packing are not deployed to the advantage of egg sales. Hence, with a new packing innovation, it may be possible to tempt the consumer with beautiful, easy-to-use egg packing to change their usual buying pattern. Other features to support this strategy

may be to reduce the price initially, to offer seven eggs for the price of six, or retail might add package of three eggs to the standard choice of egg packing.
An aesthetic, easy usable and pleasurable packing improves the beauty of product-packing combination and thus adds to the experience of the products. Nice colourful product may for example change the attention for familiar but dull package. When there are many attractive items in the same group of products, functionality of packing plays important role. The consumer may associate the sound that is heard when opening a product, with positive or negative emotions. The opening of a well known product in a new package can bring about new and wonderful feelings. Sound, colour, or light bring cognitive emotions and impact the customer to the point that he will buy products, even though they are unnecessary to him. Good light exposure of the product on the shelves, with advertency for sale and pleasant music in the shop, will relax the shopper even in the times of economy crises. After first buy of the innovative product, the customer is building trust with this design. Even if design is not perfect, the decision for the next buy is already made.

Table 1. Overview and ergonomics comparison of various egg boxes

Characteristics of egg carton box	1 	2 	3 	4 	5 
Safety and security of eggs	++++ 2 side closing	++ 2 side closing	+++ From top	+++ 1 central closing	++++ 2 side closing
Stability of box and on shelves	+++	+++	+++	+++	++
Weight	+++	++	++++	++++	+
Number of eggs	7	7	6	6	3
Production costs	++++	++++	+++	+++	++
Environment friendly	Yes	Yes	Yes	Yes	Yes

Source: author's collection from Paper Foam B.V and Albert Hein retail

Switching from unattractive packing in ordinary cartons or plastic to smooth colourful new ecological bio-based material we might bring new emotions about. With an innovation packing design, more attention is giving to details like shape, size and more peaces for lower price. For special events like Easter an extra colourful box will be attractive gimmick, especially when packed with colourfully matching fresh eggs. Looking at box from an ergonomic point of view, the box perfectly closes on two sides, and inside the box has not only good size or whole, but also the cones holders from the top insure safety and stability of eggs during their transport and storage to the shop shelves and caring them home. With redesigned packing with few open "windows" on the box, an ordinary food product like eggs, became an interesting new item (*Table 1*, *Figure 2*). Suddenly, traditionally modelled egg box, can be easy changed with a new under private label. Marketing messages can give a new imago to the simple egg box. Satisfying the first basic need of consumer for hunger, surprisingly new innovative package became competitive on the shelves. To improve sales, there is not much we can change about eggs, but there is much that we can do for the package. Good behavioural design with a motto that focuses on the target group (religious, natural and nutritionist) has to be fundamental. Once is the

design is completed, new boxes with innovative solution will encourage people to share opinion and emotions at work, home or holiday.

Egg carton construction

The standard molded egg carton structures consist of a top or cover section and the bottom cellular section. The bottom cellular section consists of different number of cells which house the eggs. These cells are arranged in parallel rows, although the number of cells and row may vary. Dozen cells arranged in two parallel rows of six cells or like in *Table 1*, *Figure 3* and *4* six cells in parallel rows of tree cells.
(www.google.com/papents/US3337110).
In new innovative bio-based carton, seven cells are placed in a round box with two rows for two eggs and in the middle row with three eggs (*Table 1*, *Figure 1* and *2*). Instead of pillars dividing the egg cells in previous well known patents, the new egg box has round shape and when two halves from the top and bottom close, they form a circle round the eggs, following their size. The last egg box in *Table 1* has three holes for eggs in a triangle shape, which is atypical for egg boxes, but handy for a single person wanting to buy eggs for a few breakfasts. Usual the structure of egg boxes is made of molded paper, paper pulp, plasterboard, plastic and thermoplastic foam such as foam polystyrene, polyethylene, polypropylene and polyvinyl chloride. The new egg carton is produced by vacuum moulding or match moulding techniques, pressing foam of pre-mix material, potato starch, fine natural fibres and water under high temperature. The egg carton has wall thickness of 2-2.5 mm, which gives sufficient protection to the eggs packed in the container and to avoid breakage egg shells. The pairs of cells from bottom and top part of the box have similar ribs and oval half egg shape, connect each pair of cells. These enable a limited amount of deformation resistance to the cellular carton section due to the forces which are applied to the carton during handling and, for example, when it is being opened and closed. On two sides the boxes has an alternative locking flange arrangement with interconnecting reinforced ribs. These enable the carton to lock and unlock or close and be reopened. Such locking arrangements have some resistance to deformation. The cell rows divide all eggs in individual cells with triangle shape support safe power portion to avoid crushing of the eggs contained therein and from compressive forces applied to the top. The new carton for seven eggs is very light in weight, so much that it is superior to ordinary carton constructions. The egg carton is strong, has a streamline design, distinguishes itself by its round edges and compact feel, and features an eye catching design available in a variety of colours. This makes the product stand out on the shelf.
The egg sample box is tasted by quality certificate compliance to regulation (EC) No 1935/2004 of the European Parliament on materials and articles intended to come into contact with dry food and may stand in contact with moist and fatty foodstuff. The product is awarded with USDA certification for bio-based material and Vinvote S349 standard for 100% bio-based and composed material.

Champagne packing innovations

Some drinks evoke memories. One of them is champagne. Bubbles in the wine with lovely one, for a birthday, a New Year eve, religious holiday, or sharing glorifying sport moments, bring our sentiments to non-rational decision. Some brands of champagne are expensive. In Europe, it will be difficult to find someone over 18 years old who has never tasted champagne. The occasions for champagne are most often connected with personal experience. For drinking French champagne or German *methode Champagnoise*, it is not necessary to visit Paris or Berlin. What is more important, during opening the bottle, moments of wellness and surprises are shared with lovely ones.

The packing of champagne plays an important role in the "champagne experience". Some people buy a bottle and wrap it in the paper to give it away. Some like it in a beautiful shopping bag. Later on the table, without appropriate shopping bag, a bottle might be less attractive. The solution might be in a user-friendly packing, which is smooth and light. An innovative champagne packing made from Paper Foam is easy to use and with hand-carry safe solution (*Figure 2* and *3*), with visual label in the middle and under. The packing material is made from a mix potato starch, natural fibber, water and pre-mix. The sludge is moulded in water and later, under the pressure and high temperature formed in the bottle shape. Two identical and symmetrical pieces (*Figure 1*), close the bottle around perfectly (*Figure 2*). In the upper and lower place of the bottle a round label is placed with a champagne or wine name printed on it. In this way, the handy packing is 100% safe, easy for carrying from shop to desirable place for consuming. The bottle stand good on the table, with an aesthetical nice shape (*Figure 3*).

Figure 1

Figure 2

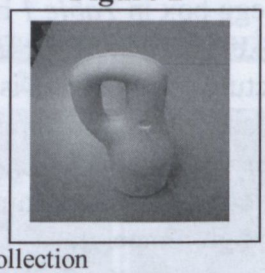


Figure 3

Source: author's photo collection

Packing has several objectives:

1) **Physical protection**-The bottle enclosed in the package may require protection from: compression, temperature, etc.;
2) **Barrier protection** – Keeping the contents clean, fresh and safe for intended shelf life is a primary function
3) **Information transmission** – Package labels communicate how to use, transport, recycle, or dispose of the package or product. Some type of information is required by governments.

Table 2. Shelves by HEMA for champagnes and wine

Ergonomic analyse of shelves by HEMA	1 	2 	3 
Placement	Entrance- in the middle of the shop on the pallet	Display-in front of the section for wine and alcohol	On the top of the shelve
Height	90-115 cm (3 layers of carton)	120-160 cm	150-180 cm
Stability	+	++	+++
Costs	+	++	+++
Weight	+++	++	+
Lifting the product	+	++	+++
Product information (price)	low	middle	high

Source: author's photo collection, place HEMA, Utrecht, 1.02.2014.

In *Table 2,* several types of shelves for champagne and wine are presented. The first picture shows three layers of original cartons, placed on the light weight plastic pallet. First lines of cartons are closed, the second are half opened and the third is open for shoppers. The champagne is easy to pick and on the comfortable height. The second shelve is a display positioned in front of all wine section on desirable height for shoppers. The last presented shelve with champagne is much higher positions, for some shoppers not easy to take and with a highest price. Champagne with distinguished looking packing can hide the price and the cheaper product might look as expensive one. The moment that is appears on the table, they certainly present a surprise. Great packing designs might help brands to survive. Design includes several disciplines like: good production solutions, marketing and estate presentation. A glass of good vine from one region can carry personal status symbol, character, trend, style and popular identical taste.

Some of these moments became portraits in the very special room on the very special place. So, who care about the price?! Everything is in innovative packing. Glass of good wine from one region can carry personal status symbol, character, trend and style, popular identical taste. It's almost impossible to share such special moments with glass of water or bier. Some champagne with a distinguee packing can hide the price and cheaper might look as expensive one. The moment that appear on the table, they certainly present the surprise. Great packing designs might help the brands to survive. Design includes several disciplines like: good production solutions, marketing, ecstatically presentation. Everything meaning of the product can be conveyed by innovative packing.

CONCLUSIONS

New innovative packing solutions reduce the amount of waste, are light in weight, and present perfect marketing tool for the possibility to sell more eggs and champagne or wine to the customers. Ecologically friendly, very demandable in western EU countries, might be solution for export similar products from Central and South East European countries to the EU market. Expanding western retail chains in the world with own standards for environment and consumers health, can offer local product in a global packing to the customers worldwide. Functional design for food packing demand that production must be in strong connection with packing and marketing and that single sample design will satisfy everyone. Packing film with perforation might help to keep fruit and vegetable fresh. The plastic tray under is an optional solution, because of the low costs and stable structure of inside of the product when transported and when on the shelves. In 21st century, laws for environment and compostable materials make distinction between functional and standards to reduce waste. The outcome is innovation in new materials that can harm our nature less and after some time to be decomposable.

In revenue packing contribute in growth for sale. The massive rush all round the world to urbanize create several problems: two or three times as many brands on the shelves make competition ferocious. Some good brands with advantageous position in the world retail market are now with new generation of consumers considered as product from the past decade. Arrival of cheep low cost travelling has made shoppers more movable and the time to "discover" a new product is shorter, compared to previous generation. Some expensive wine and cognac producers can invest little in their marketing, but they do put more efforts in new materials for packing, targeting "post-luxury" elites. Cultural background might be with mix feeling in touristic visit to France, land of champagne and wine, but on-line shoppers make world simple, clicking on their computer mouse, enabling them to buy via internet connection what they dream to taste. That put marketing of some multinational companies in weak position. New generation doesn't watch much TV. Use of smart phones

and individual search for producers in the "cloud" became so simple. Local brands commercials can be fast comparable with multinational products prices in the retails and paid by internet banking system. Young cosmopolitan generation may tech their parents to change shopping decisions, before stepping out of home.

Pleasure for shopping must be accompanied with packing innovations. Packing innovations can increase a longer shelf life for fruits and vegetables and at home use. The design of the packing however makes the product virtually enjoyable. Can we condition the consumer brain to shop with attractive packing and strong "Avatar" or metallic colours and with spoken message? Our potential consumers can be educated from start of their life to love brands for a life time. Some of them can emphasize slogans like: "House of best", "Panonia green","Blue Danuba taste", "Vajdasági yellow star".

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THE EFFECT OF BORON FOLIAR FERTILIZER ON SOME MORPHOLOGICAL PARAMETERS OF WHEAT AT DIFFERENT GROWTH STAGES

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ABSTRACT
This field research was carried out to study the effect of boron foliar application at different growth stages on morphological parameters of wheat Alex variety at Didactic Station, Timisoara, Romania during the year 2012-2013. The trial was laid out in a randomized complete block design (RCBD), consisted of three replications with the following treatments: T1 control (only received distilled water spray), T2 (B 0.3% at ZGS22), T3 (B 0.3% at ZGS41) and T4 (T2+T3) as form boric acid. Foliar solution of B was sprayed with a hand held pump sprayer at the rate of 400 L ha^{-1} on plant foliage. Experimental results revealed that the foliar application of 0.3% boron as a H_3BO_3 at different growth stages at ZGS22 and ZGS41 significantly increased plant height, spike length (cm), number of spikes m^{-2} and number of spikelets per spike which were over the control. Best results were obtained when B was applied at 0.3% at ZGS22 and ZGS41 (T4), which gave significant increase in plant height, spike length (cm), number of spikes m^{-2}, and number of spikelets per spike as compared with the control treatment. These parameters were increased by 11.11%, 15.38%, 12.81% and 17.30%, respectively.

Keywords: Boron, foliar spray application, growth stages, morphological parameters, wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an essential cereal crop, source of staple food and thus the most important crop in food security potential. More of the earth's surface is wrapped by wheat than by any additional food crop. Wheat production is the third major cereal production in the world, following maize and rice. In conditions of nutritional eating, however, wheat comes second to rice as a major food crop, known the more general use of maize as animal feed (FAO, 2013). Wheat is the second important cereal crop in Romania. It was grown on an area of 1988 thousand ha in 2012/2013 with the total production 5215 thousand metric tons with an average yield of 2.623 t ha^{-1} (INS, 2013).

Boron (B) is an important micronutrient required for plant growth, development and plays a significant role in physiological and biochemical processes. Plant needs B in small quantity. B affects not only yield but also quality of several crops. There is a wide variation in B requirement between plant species (GOLDBACH ET AL., 2001).

In several plant functions, B is disturbed directly and indirectly as it involves in cell wall formation, membrane integrity, cell wall syntheses, carbohydrate metabolism, calcium uptake, sugar transportation, flowering and fruiting, nitrogen metabolism and disease resistance (PARR AND LOUGHMAN, 1983; BONILLA ET.AL., 2009; PANDEY AND GUPTA 2013), fruit development, seed setting and grain yield is significantly responsive to B deficiency than vegetative growth (ZHANG ET AL., 1994). B deficiency is a wide-spread problem in agricultural areas world-wide, and management of B nutrition is challenged by

sudden occurrences of B deficiency or inconsistent effects of foliar B application (WIMMER AND EICHERT, 2013). Wheat plant is not sensitive to B deficiency but several studies have shown that B fertilizers both as soil application as well as foliar spraying single or shared with other micronutrients significantly improved growth, grain yield and yield components in wheat plant (AHMAD AND IRSHAD, 2011; MOEINIAN ET AL., 2011; REHMAN, 2012).

In Romania very few studies and information about the affect of foliar application of B on growth and yield productivity of wheat and is not enough documented and investigated till now.

The present investigation was carried out with a view to pursue the influence of foliar application of B on some morphological parameters of wheat at two growth stages.

MATERIAL AND METHOD

Field experiments were carried out at the Didactic Station, University of Agricultural Sciences and Veterinary Medicine "Regele Mihai I al României" din Timişoara (USAMV) during the growing season 2012-2013.

A composite soil sample was taken before beginning of the experiment from the soil surface 0-25 cm depth, air dried, ground, passed through 2 mm sieve, analysed for chemical and physical properties by using standard methods at laboratory of Physical-Chemical analysis "OSPA – USAMVB" according to (SR-ISO, 1998). Soil texture was clay having the following characteristics; sand 27%, silt 28.3%, clay 44.7%, pH 6.80, EC 0.40 dS m^{-1}, humus 3.18%, total N 2.1%, P 9.5 ppm, K 108 ppm.

The experiments was laid out in randomized complete block design (RCBD) having three replications and four foliar treatments. Seeds were sown through drills at a 15 cm distance between rows. A seed rate 270 kg ha-1 of "Alex" wheat variety was used. The unit plot size was 10.0 m x 3.0 m. A buffer zone of 2.0 m spacing was given between plots. Nitrogen was applied in two doses. First dose of nitrogen along with full dose of phosphorus and potassium were applied for all treatments at 4 weeks after sowing in the form of complex 150:100:100, respectively at the rate of 360 kg ha^{-1}. Second dose of the nitrogen in the form of urea was applied at the stem elongation stage (ZGS 30/31) at the rate 100 kg ha^{-1}. Weeds and insects associated with wheat were controlled by using a tractor - mounted boom sprayer. The B solution was applied as foliar spray to the plant leaves in form boric acid (0.3% boron w/v) at the rate of 400 L ha^{-1} with a hand pump sprayer at two growth stages of wheat. The foliar application times of B included: T1 control (distilled water spray), T2 sprayed at beginning tillering stage (ZGS22), T3 sprayed at early booting stage (ZGS41) and T4 sprayed at beginning tillering stage and early booting stage (ZGS22+ ZGS41) according to the Zadoks growth stage scales of wheat plant as described by (ZADOKS ET AL., 1974). At maturity stage, one meter square area selected randomly, selected at three locations in each plot. Plants were harvested manually and the following parameters were measured:

Plant height

The height of plants from ground level to the tip of the plant exclude spike was measured. The average height of these plants was calculated and expressed as mean plant height (cm).

Spike length

Spike length was measured from the base of first spikelets to the tip of terminal spikelets excluding awn and then average was calculated and expressed as mean spike length (cm).

Number of spikes
The number of spike was counted and then average was converted as number of spikes per square meter.

Number of spikelets per spike
The culm from the base was counted. Total number of spikelets was counted and average was recorded as number of spikelets per spike.

Data analysis
Morphological parameters were recorded and the data was analysed statistically by using the analysis of variance (ANOVA) through MSTAT-C (1991) software package. The significance of treatment means were compared by using Least Significant Difference (LSD) test at 0.05 level (STEEL AND TORRIE, 1997).

RESULTS AND DISCUSSION

Plant height (cm)
Results showed that the effect of foliar fertilizer of B on plant height was highly significant at level of 5% (*Figure 1*). The tallest plant was obtained by T4 (87.33cm) and the shortest plant was obtained by T1 (78.60cm). B plays an important role in the physiological process of plants, such as, cell elongation, cell maturation, meristematic tissue development and protein synthesis. Which in turn, maybe leads to an increase in plant height of wheat (UDDIN ET AL., 2008; AHMAD AND IRSHAD, 2011; REHMAN ET AL., 2012).

Spike length (cm)
Data regarding to spikes length are shown in (*Figure 2*). Analysis of the data showed that foliar application of B significantly affected spike length. Tallest spike length was obtained by the foliar application of B 9.15 cm (T4) and the shortest spike length was 7.93 cm (T1). Foliar application of B at ZGS22 and ZGS41 (T4) gave 15.38% increase in spike length compared to the control. This result is in agreement with KHAN ET AL., 2006, UDDIN ET AL., 2008 and REHMAN ET AL., 2012, who reported that B application significantly increase spike length.

Number of spikes per square meter
Results of this study indicated that foliar application of B significantly affected number of spike m^{-2} (Figure 3). The highest number of spike m^{-2} 405 was obtained by foliar application of B at ZGS41 (T4) and the lowest number of spike per square meter 359 was obtained from the control (T1). Number of spike per square meter increased 12.81% with ZGS22+ZGS41 as compared with control. There was no significant difference between T3, T2, and T1 treatment in number of spike per square meter. The same results have been obtained by (GUENIS ET AL., 2003; SOYLU ET AL., 2005; KHAN ET AL; 2006; ALI ET AL, 2009) who reported significant variations for number of spikes per square meter for foliar application of B.

Number of spikelets per spike
Statistical analysis of the data showed that the foliar application of B significantly increased number of spikelets per spike (*Figure 4*). Maximum number of spikelets per spike (12.00) were recorded when B was sprayed at ZGS22 + ZGS41 (T4). The minimum

number of spikelets per spike (10.23) was obtained when B was not sprayed (T1). Number of spikelets per spike increased by 17.30% as compared with control. The increase in number of spikelets per spike may be due to the reason that B plays a vital role in flowering and grain setting of wheat. These results are in agreement with the finding of UDDIN ET AL. (2008) and REHMAN ET AL. (2012) who reported that B application increased number of spikelets per spike in wheat.

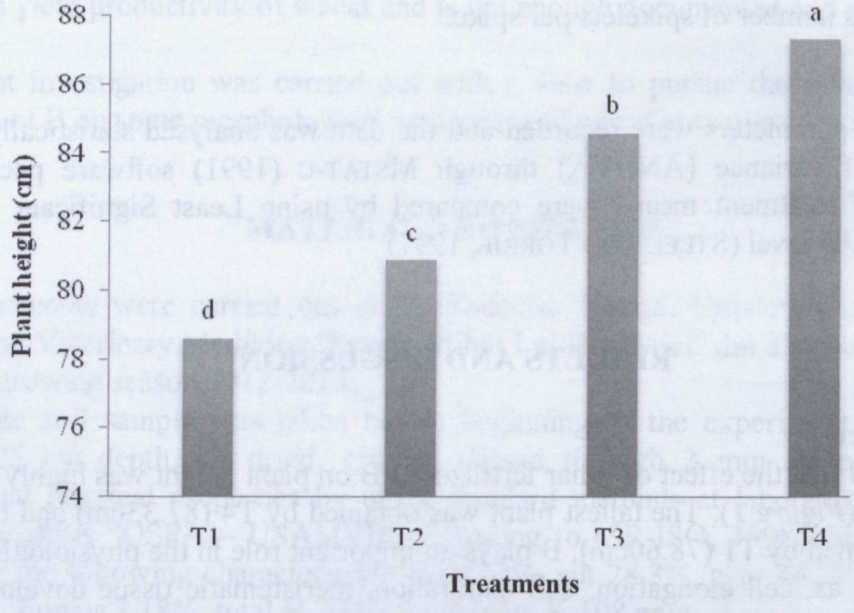

Figure 1. Effect of foliar fertilizer of Boron at different growth stages on wheat plant height.

The columns sharing the same letter are not significantly different at level of P = 0.05

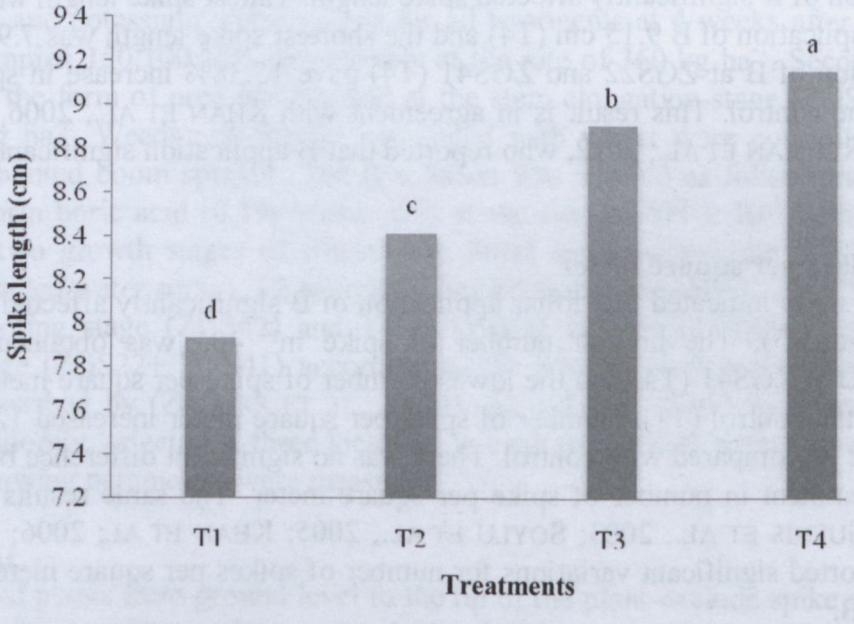

Figure 2. Effect of foliar fertilizer of Boron at different growth stages on spike length of wheat plant.

The columns sharing the same letter are not significantly different at level of P = 0.05

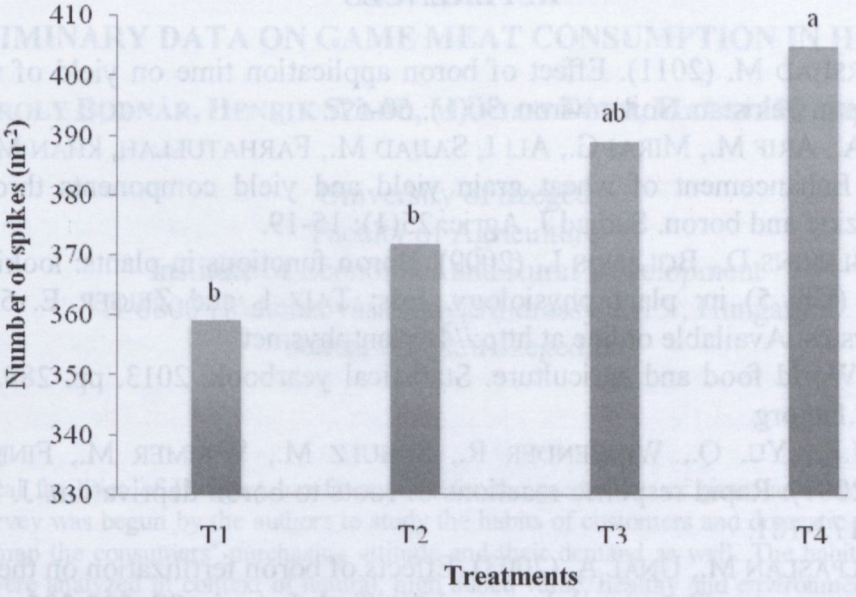

Figure 3. Effect of foliar fertilizer of Boron at different growth stages on number of spike m^{-2} of wheat plant.

The columns sharing the same letter are not significantly different at level of P = 0.05

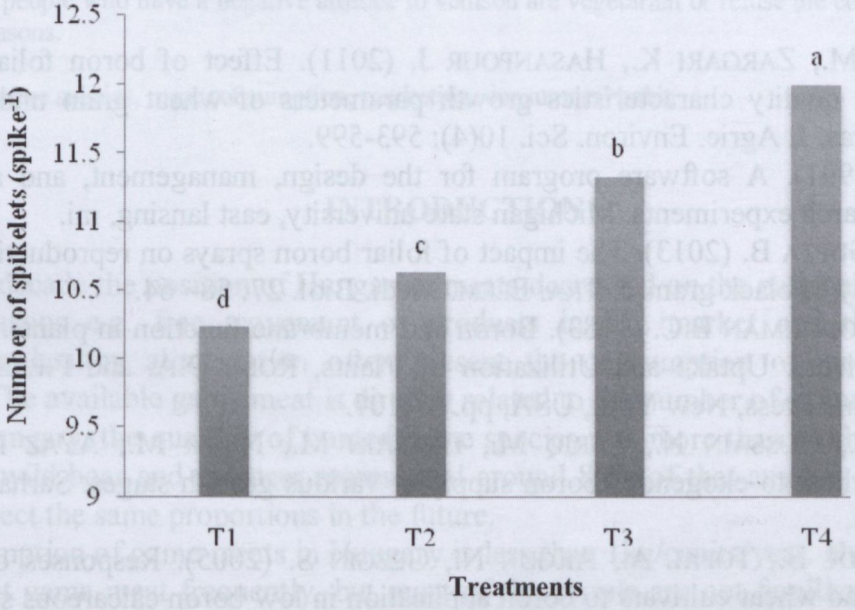

Figure 4. Effect of foliar fertilizer of Boron at different growth stages on number of spikelets per spike of wheat plant.

The columns sharing the same letter are not significantly different at level of P = 0.05

CONCLUSIONS

The current study showed that foliar application of Boron (0.3%) at different growth stage at ZGS22 and ZGS41 significantly increased wheat plant height, spike length (cm), number of spikes m^{-2}, and number of spikelets per spike. The highest values of all studied parameters were obtained from T4 treatment. The control (T1) gave the lowest values of all studied parameters. For getting more general results, it is recommended to repeat the experiment in other locations.

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PRELIMINARY DATA ON GAME MEAT CONSUMPTION IN HUNGARY

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ABSTRACT

The ecological facilities of Hungary are favourable for the production of game animals and venison. An extensive survey was begun by the authors to study the habits of customers and domestic consumption. The aim was to map the consumers' purchasing attitude and their demand as well. The habits and attitudes of consumers were analyzed in context of natural, high added value, healthy and environment friendly animal products: the meat of game animals.

Data were collected by questionnaires. The results represent a descriptive picture on the acceptance, rejection, attitudes and preferences concerning the given product categories. In the recent study we focused on the following species: red deer, fallow deer, roe deer, wild boar, hare, pheasant and mallard duck.

Differences were found between the answers of the asked sample population living in cities and in the rural areas. Those people who have a negative attitude to venison are vegetarian or refuse the consumption due to emotional reasons.

Keywords: game species, meat consumption, marketing, consumers' habit

INTRODUCTION

In the last decade the position of Hungarian meats decreased on the national market due to several reasons e.g. free movement of products in EU market and the actions for propagating healthy alimentation often present the consumption of meat in negative meaning. The available game meat is directly related to the number of animals shot in each year. In Hungary, the quantity of hunted game species was more than 10 thousand tons in 2012, and wild boar and red deer represented around 80% of that amount. BLEIER ET AL. (2013) expect the same proportions in the future.

The consumption of game meats in Hungary is less than 1kg/capita/year. Hunters and their families eat game meat frequently, but most of the people are not familiar with it (GFK, 2003). (The Central Statistical Office uses the COICOP system of the European Union for the collection of data and calculates the average consumption value of main food categories for the statistical regions (ABONYINÉ PALOTÁS AND KOMAREK, 2004).)

The quality of the product has a great influence on consumption. Product-oriented quality, process-oriented quality and quality control can also be said to constitute objective quality. User-oriented quality can be said to constitute subjective quality, since it can be measured only at the end-user, and can differ for the same product between users. User-oriented quality can also be influenced by factors that are not characteristics of the product itself, such as the purchase situation, type of retail outlet, price, brand, etc. Much of the discussion on quality in the food industry is concerned with product and process-oriented quality and quality control, while the consumer evaluates and pays for subjectively perceived quality. The amount a consumer is willing to pay for a product depends on this subjectively perceived quality, which is related to, but not the same as, objective quality. Improvements in objective quality, which have no effect on consumers' perceived quality

will have no commercial effect, and hence no positive effect on the producer's competitive situation (BRUNSØ ET AL., 2002).

Generally not only the individuals' economic and socio-cultural status determines the nutritional habits, but also the other way round: food consumption could be used to predict social and economic status as well as key values and value judgements. Value judgements as reflected in nutrition are analysed at the level of the consumers' general value systems, values influencing consumption habits, and the motives for selecting particular products. The importance of the traditional cooking habits is decreasing step by step on weekdays, and eating became satisfaction of requirements without formalities for a part of consumers. At the same time they are looking for the traditional styles of nourishment as sources of experiences. The classification of consumers can be done by several different ways. One part of the people would like to have special meals and eating out (gourmet), while the semi-finished or ready-made products (e.g. fast-food) are preferred by others. The health-conscious groups are seeking for fresh and natural (organic) foodstuff as guarantee of health or trust in high-tech based products (HORVÁTH ET AL., 2005).

In the rural area, meat and meat products are the foods most demanded: they are eaten twice or three times a week. In the urban area, meat and meat products consumption occurs four times a week, but there are also people that do not eat any meat at all for various reasons (health, fashion, etc.). No matter the area of origin – rural or urban – meat and meat products are considered basic foods by most respondents (PETROMAN ET AL., 2013). HORVÁTH AND SOÓS found (2007) that the consumers are ready to taste and buy new meat products, e.g. new fish species became successful in a relatively short time.

The aim of our research is to map the consumer requests, attitudes and preferences concerning the game meat market. The goal of the recent study was to optimize the questionnaire and the research methods by the experiences of the first 250 interviews.

MATERIAL AND METHOD

Recent study is a part of a wide spread survey on venison consumption pattern of Hungarian consumers. The survey was carried out by on-line and paper based questionnaire as well. During the survey people were asked (n=250) about their attitudes to eating and shopping habits of game meats. The population segment above 18 years of age had equal chances to get into the group of interviewees; however the students who conducted the interviews a little bit focused on the younger target group. Due to the relatively small size of the sample group the compositions of the data were distorted, so the survey can not be regarded as a representative one, but the data of further study will be corrected, when the size of sample will increase.

The questionnaire contained mostly closed questions, in some cases interval scale was applied. To make some answers more exhaustive free contextual answers could also be given. The questions were focused on the following areas:

- personal information about respondent people (sex, age, level of qualification, hunting activity),
- opinion about game meat consumption (consumption pattern, causes of preference or rejection), the frequency was scaled with intervals: per year, per half year, in every 3 months, in every month, every week;
- opinion about different game meats (preference of species: mallard duck, pheasant, hare, roe deer, wild boar, mouflon, fallow deer, red deer; opinion value of delight,
- places of purchasing.

(Further data will be processed later.)

Data obtained were submitted to statistical analysis by using PASW Statistics 18 software package. Results were expressed as proportions and frequency distributions of the analyzed sample.

RESULTS

As shown in *Table 1* the distribution of the target group, there was more women than man and younger than elder people among the interviewees. By the type of activity 159 persons had intellectual work, and 86 of them had manual labour (5 persons did not answer). Most of the sample group has high school and university degree as level of education (*Table 2*).

Table 1. The age and gender distribution of the responders (capita)

Age (years)	Male	Female	Total
18-24	69	80	149
25-34	24	24	48
35-44	12	18	30
45-60	10	10	20
Above 60	1	2	3
Total	116	134	250

Table 2. The distribution of the responders by their level of education (capita)

Gender	Level of education	Age (years)					Total
		18-24	25-34	35-44	45-60	60<	
women	Primary school	1	0	2	0	1	4
	High school	63	11	6	5	0	85
	College/University	4	12	4	5	0	25
Total		68	23	12	10	1	114
men	Primary school	1	1	0	0	0	2
	High school	73	14	9	4	0	100
	College/University	4	9	9	5	2	29
Total		78	24	18	9	2	131

Table 3. Attitude to eating game meat (capita)

Gender	Consumption	Age (years)					Total
		18-24	25-34	35-44	45-60	60<	
women	Ate already	60	23	12	10	1	106
	Never tasted	8	0	0	0	0	8
Total		68	23	12	10	1	114
men	Ate already	76	24	18	9	2	129
	Never tasted	1	0	0	0	0	1
Total		77	24	18	9	2	130

Both of the groups of ladies and men over the age of 25 year ate already game meat in 100% (*Table 3*). Under the age of 25 years 12% of the ladies and 1.3% of the men have not tasted the game meats yet.

The hunters are over represented in the survey (*Table 4*), because only 0.5% of the Hungarian inhabitants has hunting licence.

44.1% of the consumers (*Table 5*) used to eat game meat less frequently than once in a year. Only 4.6% of the interviewees eat venison every week, and of course all of them is hunter. High rate of people eat game meat, but most of them only at celebrations or at special occasions, except the hunters who prepare game almost at every weekends.

Table 4. Attitude to eating game meat (capita)

Gender	Consumption	Age (years)					Total
		18-24	25-34	35-44	45-60	60<	
women	Hunter	2	5	0	0	0	7
	Non-hunter	66	18	12	10	1	107
Total		68	23	12	10	1	114
men	Hunter	18	11	13	7	2	51
	Non-hunter	60	13	5	2	0	80
Total		78	24	18	9	2	131

Table 5. Frequency of game meat consumption (capita)

Frequency of consumption	Age (years)					Total
	18-24	25-34	35-44	45-60	60<	
Once a week	5	1	2	2	1	11
Once a month	20	13	10	5	0	48
Once in 3 months	20	8	4	4	0	36
Once in half year	21	1	2	1	0	25
Once a year	8	3	2	0	0	13
Less frequently	65	21	10	7	2	105
Total	139	47	30	19	3	238

Only 19 persons from 250 respondents (*Table 6*) have problem with the eating meat and/or game meat. The most frequent reason for the rejection is the emotional reason, but some of the answers show that some people do not know game meats and some do not know where to buy it. Only one person was afraid of the possible zoonotic diseases.

2/3 part of the consumers (*Table 7*) get the game meat directly from those who are authorized for hunting. 30.3% of the consumers buy it at meat shops, and only 3.3% looking for venison in hypermarkets. The types of meat (the preferred species) were chosen by most of the consumers on the price, quality and appearance (*Table 8*).

Table 6. Reasons for rejection of game meat consumption (capita)

Reasons for rejection	Age (years)					Total
	18-24	25-34	35-44	45-60	60<	
Vegetarian	3	0	0	0	0	3
Do not know this meat	5	0	0	0	0	5
Emotional reasons	5	1	0	1	1	8
Don't know where to buy	1	1	0	0	0	2
Hygienic risk	1	0	0	0	0	1
Total	15	2	0	1	1	19

Table 7. Reasons for rejection of game meat consumption (capita)

Source of supply	Age (years)					Total
	18-24	25-34	35-44	45-60	60<	
Hypermarket	4	1	1	1	1	8
Meat shop/butcher	48	11	8	5	2	74
Directly from hunter	94	34	21	13	0	162
Total	146	46	30	19	3	244

Table 8. Willingly consumed meats (capita)

Species	Age (years)					Total
	18-24	25-34	35-44	45-60	60<	
Mallard duck	4	0	0	0	0	4
Pheasant	28	7	5	1	0	41
Hare	7	0	0	0	0	7
Wild boar	58	19	14	13	2	106
Roe deer	41	14	8	4	0	67
Mouflon	2	0	0	1	1	4
Fallow deer	2	2	0	0	0	4
Red deer	4	4	3	0	0	11
Total	146	46	30	19	3	244

CONCLUSIONS

Game meat is usually described as healthy and natural food and its consumption has a good effect on human nutrition and physiology.

Most of the people among the interviewees ate already or used to eat game animals. Almost all hunter men ate every species, but most of the people choosed only one, which is their favourite, easy to get and/or cheap. Almost all of the pheasants and hares were coming directly from hunters or their families and friends as fresh meat in skin to the table of consumers. The distribution of the preferred game species was coming from the characteristic of the region. All the consumers described the game meat healthy and natural sources of protein and minerals, but the answers during the interviews about ingredients (protein, fat and mineral content) of the meats were confused, and the number of inadequate responds demonstrated insufficient information on this field. Probably the publication of scientific data on characteristics of the frequently consumed meats, e.g. wild boar meat (BODNÁRNÉ SKOBRÁK ET AL., 2008) could improve the situation.

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LAMB FATTENING POSSIBILITIES IN MIXED FLOCK OF SHEEP

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ABSTRACT

The aim of this study was to determine daily weight gain of different Würtemberg crosses. The experiment was carried out in central part of Vajdaság province, at right and left side of river Tisza in Bácska and Bánság region. In flock of 240 Würtemberg ewes 3 genotype of ram were used: Würtemberg, Ile de France and Charolais. Trial included 60 lambs of three breed and crosses, 20 lambs per each: I. group pure Würtemberg, II. group Ile de France x Würtemberg and III. group Charolais x Würtemberg. Average body mass of lambs at the beginning of trial was approx. 12 kg and at the end of the trial approx. 30 kg. The lambs were divided into two groups: indoor and pasture trial group. Lambs of group A were kept exclusively on pasture for fattening, fed by mothers milk and grass. Lambs of group B were kept indoors fed by mothers milk, concentrate mixture and alfalfa hay (ad libitum). During the experiment all lambs in group had same housing and nutrition condition. At the indoor condition the average daily gain of pure Würtemberg breed were 290 g for ram and 279 g for ewe lambs. In the case of Ile de France x Würtemberg crosses the daily gain were 313 and 309 g respectively. Charolais x Würtemberg crosses showed daily gain of 333 and 300 g per day. In this case the ram and ewe lambs obtained the best daily gain in the group. In the pasture condition the crosses of ram the Charolais x Würtemberg show the highest results 271 g while in the case of pure Würtemberg breed the result was 226 g/day. The average values of Ile de France x Würtemberg crosses are in the middle with daily gain value of 250 and 243 g/day. Würtemberg breed and their crosses at indoor condition have realised higher daily gain average. This confirms the fact that in crossing beside the genetic difference between populations that are being crossed, important factor for better daily gain is also the system of feeding. At indoor and also in outdoor condition the all breed crosses of Charolais x Würtemberg lambs got the highest daily gain results. In that case of crossing the Charolais breed has an outstanding effect.

Key words: lamb fattening, Würtemberg, Ile de France, Charolais breeds

INTRODUCTION

Sheep are the most efficient converters of the hundreds of thousands of hectares of marginal vegetation into high quality animal protein (FLAMANT AND MORAND-FEHR, 1982; BOYAZOGLOU ET AL., 1985; BOYAZOGLOU AND FLAMANT, 1990). Today, there are many sheep populations and different rearing systems, which are conditioned by natural and economical factors and sheep producing tradition in certain countries or regions. Therefore, there is no general model which could apply for all farms and conditions (OSAMU ET AL., 2005; PETROVIC, 2007). Researchers are facing the responsibility to define existing systems and point out to directions of future development of sheep breeding (ALMAHDY ET AL., 2000; VIZARD AND NIVEN, 2002; UGARTE, 2007). The fattening operations are among the important activities within sheep production sector. The variations are due to several reasons; among these are the sizes of investments, location of

the fattening farm and the experience of farmers. Locally, there are two types of lamb fattening systems, the commercial – intensive (rarely used) and the semi-extensive systems. In the first, commercial fattening manufactured feeds are used in these operations while the pasture-based diet is used in the semi-extensive system, (HAMMAD ET AL., 2002). The system of raising lambs for meat under grazing with supplementation although is cost effective, the procedure has not been largely adopted by the farmers due to their poor economical background and age old traditional practices. Low production of mutton and lamb meat is consequence of numerous factors such as: poor breed structure, inadequate nutrition and primitive breeding methods, (RUZIC-MUSLIC ET AL., 2005). Analysis of sheep production systems in the world shows that in many countries slaughter lambs are crosses in 30-70% of cases (MITIC, 1984). The objective of this study was to compare different lamb crosses in different feeding systems.

MATERIAL AND METHOD

The experiment was carried out in central part of Vajdaság province, at right and left side of river Tisza in Bácska and Bánság region. In flock of 240 Würtemberg ewes 3 genotype of ram were used: Würtemberg, Ile de France and Charolais. Trial included 60 lambs of three breed and crosses, 20 lambs per each: I. group pure Würtemberg, II. group Ile de France x Würtemberg and III. group Charolais x Würtemberg. Average body weight of lambs at the beginning of trial was approx. 12 kg and at the end of the trial approx. 30 kg. The lambs were divided into two groups: indoor and pasture trial group. Lambs of group A were kept exclusively on pasture for fattening, fed by mother's milk and grass. The animals were left to graze from 8:00 to 18:00 h every day, had free access to fresh water and received no supplementary feed. Lambs of group B were kept indoors fed by mother's milk, concentrate mixture (*Table 1*) and alfalfa hay (*ad libitum*). During the experiment all lambs in group had same housing and nutrition condition. Control measuring was carried out in 7 day intervals using standard zoological methodology. Obtained results on monitored parameters were processed using SPSS statistical program package.

Table 1. Structure and nutritious value of the concentrate mixture in %

Components	Ratio
Corn	45,0
Wheat	20,5
Soybeen grits	16,0
Sunflower cake	6,0
Yeast	3,0
Alfalfa meal	7,0
Salt	0,5
Pre mixture	2,0

RESULTS

Data on production parameters of lambs at indoors condition according to crosses is presented in *Table 2*. The average daily gain of pure Würtemberg breed were 290 g for ram and 279 g for ewe lambs. In the case of Ile de France x Würtemberg crosses the daily gain were 313 and 309 g respectively. Charolais x Würtemberg crosses showed daily gain of 333 and 300 g per day. In this case the ram and ewe lambs obtained the best daily gain in the group.

Table 2. Production results of lambs in fattening at indoors condition

Breed or crosses	n	Average daily weight gain (g/day)	
		ram	ewe
Würtemberg	20	290	279
Ile de france x Würtemberg	20	313	309
Charolais x Würtemberg	20	333	300

Data presented in *Table 3* show the lambs daily weight gain at pasture condition. The crosses of ram the Charolais x Würtemberg show the highest results 271 g while in the case of pure Würtemberg breed the result was 226 g/day. The average values of Ile de France x Würtemberg crosses are in the middle with daily weight gain value of 250 and 243 g/day. Concerning above mentioned breeds and crosses all presented daily weight gain results are very similar with other authors experiment records, (PLATT, 2006; GOLDING ET AL., 1976; RAICHEVA ET AL., 2007).

Table 3. Production results of lambs in fattening at pasture condition

Breed or crosses	n	Average daily gain (g/day)	
		ram	ewe
Würtemberg	20	226	219
Ile de France x Würtemberg	20	250	243
Charolais x Würtemberg	20	271	261

DISCUSSION AND CONCLUSIONS

According to the presented result, Würtemberg breed and their crosses at indoor condition have realised higher daily gain average (*Table 2*). This confirms the fact that in crossing beside the genetic difference between populations that are being crossed, important factor for better daily gain is also the system of feeding. It is evident that beside of obvious effect of heterosis the way of feeding resulted in higher daily gain and body weight. Researches by other authors have confirmed our statement (PETROVIC ET AL., 1998; CAMERON AND DRURY, 1985).

But from the financial point of view the additional feeding investments like using the concentrate mixture as well as the alfalfa hay are not always subservient. Practically there is no common model which could apply for all rearing system, (RAICHEVA AND IVANOVA, 2007). In sheep production in Serbia, in the last several decades, there have been some changes in the sheep rearing system. Conditions of nutrition and care have been improved, and local populations have been improved not only through selection measures but also planned or unplanned crossing with foreign breeds. Also, foreign breeds have been imported, and some of them succeeded to adapt to new conditions and are reared in pure breed, (PETROVIC ET AL., 2009.). But answer for the question that potentials of different breed crossing and production in different rearing systems, to decide which will serve better the defining direction, will be obtained only by experiments under the given circumstances.

By the other hand our results indicate the importance of ram type in crossing (PETROVIĆ, 2000; VIZARD AND NIVEN, 2002; OSAMU ET AL., 2005). Namely at indoor and also in outdoor condition the breed crosses of Charolais x Würtemberg lambs got the highest daily gain results. It was the same in the case of ram and ewe lambs. In that case of crossing the Charolais breed has an outstanding effect.

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RECENT EXPERIENCE OF BIOREGIO CARPATHIANS PROJECT: IUCN CATEGORIZATON OF SPECIES OCCURING IN THE CARPATHIAN REGION OF HUNGARY

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ABSTRACT

In conservation biology increasingly more countries are using the IUCN categorization (IUCN category and criteria) to describe species occurrence and population dynamics. Although, the evaluation process of the categories is strict, the category and criteria might change, due to different spatial levels (continent, country, region), which often provide basis for considerable debates, like in the BioRegio Carpathians SEE. The basic aim of the program is to manage and conserve protected areas and natural resources in the Carpathian region, therefore increase the attractiveness of the area. Our aim with this study is to demonstrate the difficulties of the assessment and to draw attention to potential pitfalls. We have evaluated IUCN categories for 46 fish, 13 mammal and four bird species. Based on our result we can state that we usually do not *even* have enough verified data to evaluate the exact IUCN categories for more studied and well known taxa like mammals or birds. Primary reason for this is the lack for sufficient data (area, population size, population decrease/increase) that is needed for an accurate evaluation.

Keywords: nature conservation, IUCN categories, Bioregio Carpathians, databases, red list

INTRODUCTION

In conservation biology increasingly more countries are using the IUCN categorization (IUCN category and criteria) to describe species occurrence and population dynamics. Although, the evaluation process of the categories is strict, the category and criteria might change, due to different spatial levels (continent, country, region), which often provide basis for considerable debates. The primary reason for this debate is that we usually do not have well controlled and verified public databases. However, these databases are essential to assign species in accurate IUCN categories and criteria. Owing to this, the evaluation process is usually aided by individual experts and their educated guesses. We have found the same situation during our work in a recent project, the BioRegio Carpathians SEE, which started in 2012. There are seven countries involved in this international project, all shares a different proportion from the Carpathian region (*Figure 1*). The participating countries are as follows: the Czech Republic, Poland, Hungary, Romania, Serbia, Slovakia and Ukraine. The basic aim of the program is to manage and conserve protected areas and natural resources in the Carpathian region, therefore increase the attractiveness of the area. BioRegio seeks to develop common standards in integrative management, so that countries can apply joint management and implementation techniques. Another important objective of the program is to create a common biodiversity information system and to prepare and maintain a red list of threatened species and habitats, based on existing databases. There is a great importance in the project for exploring and resolving legal, social, economic, and natural problems and barriers. In BioRegio Carpathians SEE, classification of the threatened species and habitats are based on international IUCN categories (IUCN 2012).

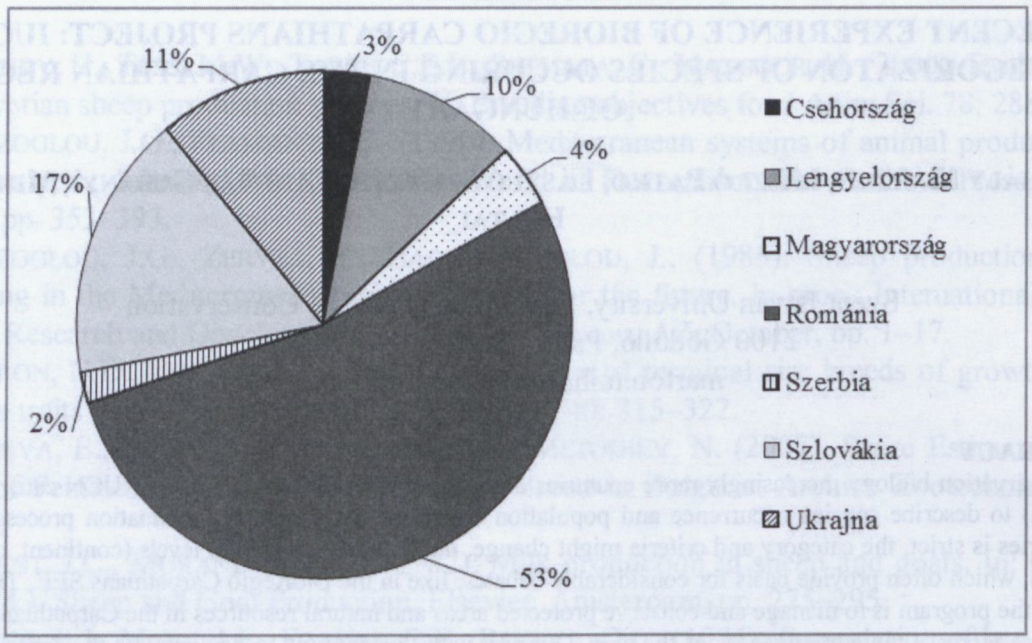

Figure 1. Regional share of the participating countries in BioRegio Carpathians SEE project

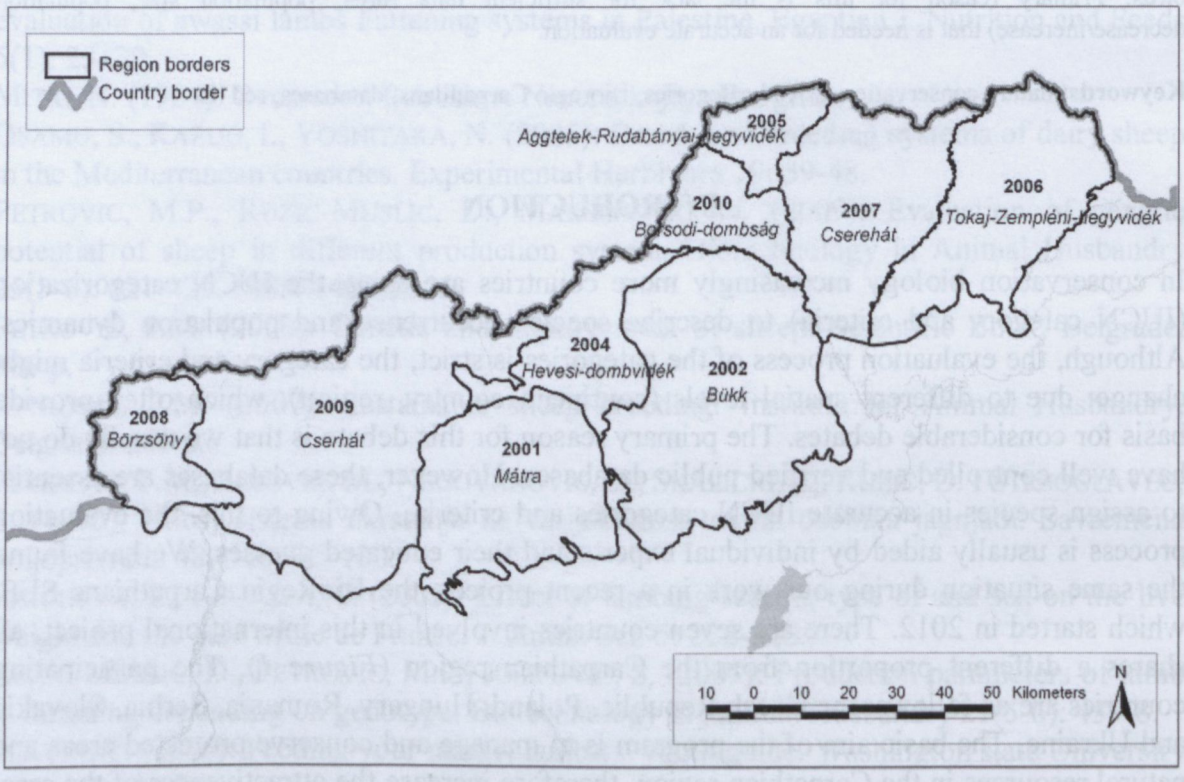

Figure 2. Orographic regions designated in the Carpathian region of Hungary

During the program, our first task was to evaluate the occurrence, threatening factors and conservation approaches for protected and invasive species in all nine orographic regions (*Figure 2*). For this we tried to gather information from public and easily accessible databases, but we could not find any. Number of species in different phylum was as follows: molluscs 61, arthropods 675 vertebrates 505 and other 5 species (total of 1246 species). The second task was to evaluate the same information in given taxonomic

categories (dragonflies (56 species), spiders (1075 species), fish (63 species), molluscs (417 species), altogether 1611 species. These species were categorized as protected in other countries but not in Hungary.

Evaluating natural values based on IUCN categories and criteria is becoming increasingly important in our country too. Our aim with this study is to demonstrate the difficulties of the assessment and to draw attention to potential pitfalls. For this reason, we have chosen several different species where we could find basic data for evaluation.

MATERIAL AND METHOD

We have categorized 46 fish, 13 mammal and four bird species [IUCN categories and criteria (IUCN 2012)]. Mammal and bird species data were given in national level, but fish species IUCN categories and criteria were evaluated at Carpathian level. We have done all evaluation based on literature references. In case of fish we compared four individual experts opinion, whom contributed to BioRegio Carpatians SEE, with literature data. Fisher's exact test was used for statistical analysis (FISHER, 1922; REICZIGEL ET AL., 2010). The calculated IUCN categories of the other two taxa were compared to Hungarian legal status (protected, strictly protected or huntable) *(Table 1)*.

In case of fish, classification was considered identical if expert's opinion were the same with literature based IUCN categorization. In case of mammals and birds the following compliance were made: LC = huntable, NT = protected, and VU, EN, CR = strictly protected.

Table 1. Those IUCN categories that this study dealt with and their descriptions (IUCN 2012)

Categories	Abbreviation	Description
Critically Endangered	CR	A: population decrease in the past ≥ 90% B: Geographic range < 100 km^2
Endangered	EN	A: population decrease in the past ≥ 70% B: Geographic range < 5000 km^2
Vulnerable	VU	A: population decrease in the past ≥ 50 % B: Geographic range < 20000 km^2
Near Threatened	NT	Taxon has been evaluated against the criteria but does not qualify for CR, EN or VU now, but is likely to qualify for a threatened category in the near future.
Least Concern	LC	Widespread and abundant taxa are included in this category.

RESULTS

In case of fish species, we have found several differences between experts' opinion and calculated IUCN categories *(Table 2)*. When we compiled all fish species, we have found that experts opinion significantly differ from IUCN classification. If we focus only on protected species we can see that only first and second experts' opinion differed. Categories of not protected species never showed statistical differences.

Table 2. Comparison of experts' opinion with IUCN classification

Fisher's exact test	All species		Protected species		Non protected species	
	p	n	p	n	p	n
Expert 1	**0.004**	92	**0.024**	36	0.172	56
Expert 2	**0.001**	92	**0.008**	36	0.274	56
Expert 3	**0.033**	92	0.219	36	0.149	56
Expert 4	**0.020**	92	0.084	36	0.303	56

Significant differences were shown in seven taxa. The species, *Eudontomyzon danfordii* were categorized as endangered by all four experts, but our literature (HARKA AND SALLAI, 2007; HALASI-KOVÁCS AND HARKA, 2012) based IUCN classification suggest that it should be near threatened. Three experts evaluated *Gymnocephalus schraetser* and *Rutilus virgo* as endangered species, while one expert wrote they are vulnerable. Based on publications both species were assigned in near threatened category. In case of *Umbra krameri* two experts' opinion was that the species is endangered and two experts wrote that it is vulnerable. This species should also be only in near threatened category. One expert's opinion is that *Carassius carassius* should be endangered, three others said it should be vulnerable. According to literature data this species is also near threatened. *Phoxinus phoxinus* and *Zingel zingel* said to be vulnerable, but based on literature they should only be near threatened. The cited publications mention population decrease (HARKA AND SALLAI, 2007; MÜLLER ET AL., 2011) but they do no mention the rate of decrease. That is why categorization only meets the criteria for near threatened but not for endangered or vulnerable.

In case of mammals and birds, most species (76.5%) IUCN category and Hungarian classification is the same. Although, three species shown excessive difference *(Table 3)*. *Lepus europaeus* and *Perdix perdix* are huntable species, however their nationwide classification based on literature should be vulnerable and critically endangered. *Lutra lutra* is a strictly protected Carnivore in Hungary, but due to its stabile area and increasing population (HELTAI, 2010; HELTAI ET AL., 2012) it should be in least concern category.

Table 3. Hungarian legal status of some mammal and bird species and their IUCN categories based on literature

Scientific name	Hungarian name	Status	Calculated IUCN categories	Bibliography
Felis silvestris	Vadmacska	Strictly protected	NT	HELTAI 2010
Lynx lynx	Hiúz	Strictly protected	CR (D)	HELTAI 2010
Canis lupus	Szürke farkas	Strictly protected	CR (D)	HELTAI 2010
Lutra lutra	**Vidra**	**Strictly protected**	**LC**	**HELTAI ET AL. 2012**
Perdix perdix	**Fogoly**	**Huntable**	**CR (A1ce)**	**CSÁNYI ET AL. 2013**
Cervus elaphus	Gímszarvas	Huntable	LC	CSÁNYI ET AL. 2013
Capreolus capreolus	Őz	Huntable	LC	CSÁNYI ET AL. 2013
Sus scrofa	Vaddisznó	Huntable	LC	CSÁNYI ET AL. 2013
Lepus europaeus	**Mezei nyúl**	**Huntable**	**VU (A1ce)**	**CSÁNYI ET AL. 2013**

CONCLUSIONS

Our results showed that significant differences can be observed if we compare Hungarian legal status or experts' opinion with calculated IUCN categories based on publication and literature data. From 48 fish species eight were considered as endangered or vulnerable. From this eight species, nor could meet the criteria for any higher risk categories (VU, EN, CR). From the 17 mammals and birds species three were controversially *(Table 3). Lutra lutra* population is increasing in Hungary (HELTAI ET AL., 2012), therefore it should be in least concern category. Protection might be justified based on vulnerable wet habitats (FARAGÓ, 2008), although strictly protected status may lie on emotional basis and not on exact data. Another controversial species is *Perdix perdix*. Its population has massively decreased from the 1970s (FARAGÓ, 2000; CSÁNYI ET AL., 2013). Owing to the more than 90% population decrease *Perdix perdix* should be critically endangered. In Hungary this species is huntable, but only where individual release take place and just at the release site [72/2012. (VII. 24.) VM decree]. The third species is *Lepus europaeus*, which still has a great importance for wildlife management. In the last 50 years population decrease (FARAGÓ, 2006; CSÁNYI ET AL. 2013) was higher than the bottom limit for vulnerable category. In 2012, hunting season on *Lepus europaeus* was shortened by one month [72/2012. (VII. 24.) VM decree]. During the data collection period of BioRegio Carpathians SEE no information was gathered from the last two species. The reason for this is nor *Perdix perdix*, neither *Lepus europaeus* are protected in Hungary. Based on our result we can state that we usually do not *even* have enough verified data to evaluate the exact IUCN categories for more studied and well known taxa like mammals or birds. In the early stage of this study we tried to find public and easily accessible databases, but we could not find any. The reason for that is databases that collects information on a regular basis on given species groups do not exist, except from few examples like Hungarian National Game Management Database. Database to organize historical data are also seldom. Therefore IUCN categorization becomes impossible to evaluate. That is why international project coordinators search for individual experts of a given species or taxon who can guess the category based on his/her expertise. However, after this, the categorization become hardly or not verifiable, and also strongly depends on the experts' knowledge. Thus, one of the most important and fundamental aspect of science will be questioned; the reproductivity. Furthermore, in the case of BioRegio Carpathians SEE classification should have been done in more detailed level (e.g.: region) than national. That leads us to a need for more developed databases where regional sampling can be carried out. Within the framework of BioRegio for us it was clear that Hungary is not the only one with this problem. This raises the question: how reliable is the collected data that international projects base on? These questionable databases can give solid ground for species conservation plans and can also aid decision makers. To solve this problem, we would need supported monitoring programs with sufficiently unified methodological background that are suitable for scientific publication. In our country continuously operating monitoring systems with a national level coverage like Hungarian Biodiversity Monitoring System (http://www.termeszetvedelem.hu/nbmr) and Hungarian Nature Conservation Information System (http://geo.kvvm.hu/tir/viewer.htm) should collect information on other species than just protected ones.

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UTILIZATION OF OILSEED RESIDUES AND OAT BRAN AS SUBSTRATES FOR β-GLUCOSIDASE PRODUCTION BY ZYGOMYCETOUS FUNGI

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ABSTRACT

Utilization of agro- and food-industrial residues has special importance today. One possible way of the applications is the production of industrially interesting microbial hydrolases, for which, solid-state fermentation is a low-cost and environmental friendly biotechnological technique. Since most filamentous fungi are able to biodegrade the cellulosic content of these natural materials, high-yield production of extracellular β-glucosidases can be obtained during the fermentation. In this study, zygomycetous fungal strains representing *Rhizomucor miehei*, *Rhizopus stolonifer*, *Mucor corticolus*, *Mortierella echinosphaera* and *Umbelopsis autotrophica* were grown in solid cultures containing various oilseed residues and oat bran as carbon sources to assess the production of their β-glucosidases. Among the oilseed residues, pumpkin seed proved to be the best inductor for β-glucosidase activity. Enzyme production of *R. miehei* and *U. autotrophica* was further enhanced when oat bran was used as support. Effect of minerals salts on the enzyme yield was also assayed, in which β-glucosidase production of the investigated strains was generally stimulated after moisturizing the substrates by mineral salt solution.

Keywords: plant residues, filamentous fungi, solid-state fermentation, extracellular β-glucosidase

INTRODUCTION

Agricultural and food industrial processes produce large amount of by-products and waste materials which can be utilized for various microbiological-biotechnological applications. Besides the bioethanol preparation (SARKAR ET AL., 2012), high-yield production of microbial enzymes is also useful direction to exploit these substrates efficiently. Since filamentous fungi can produce a variety of hydrolases (e.g. cellulases, lipases, proteases), fermentation using these microorganisms is a well applicable method for decomposing the plant-derived materials. As part of the cellulase enzyme system, β-glucosidases (EC 3.2.1.21) play crucial role in the enzymatic hydrolysis of cellulosic content (GAO ET AL., 2013; SINGHANIA, 2009). These enzymes catalyze the hydrolysis of cellodextrines as well as the endo- and exoglucanase inhibitor cellobiose to glucose (LYND ET AL., 2002). The hydrolyzing activity of β-glucosidases can be utilized in various applications, such as enzymatic degradation of olive wastewater, or liberation of aroma and antioxidative phenolic compounds from plant-derived products (KHOUFI ET AL., 2011; ACOSTA-ESTRADA ET AL., 2014). Under certain conditions, β-glucosidases are able to transfer glycosyl groups to saccharides and alcohols resulting in the formation of pharmaceutically important oligosaccharides, alkyl-glycosides, and different glycoconjugates (SMAALI ET AL, 2007).

Zygomycetes fungi are a remarkable group of filamentous fungi. Many species, especially those belonging to the order Mucorales, have successfully been used for production of cellulase enzymes (FERREIRA ET AL., 2013). However, compared to other filamentous

fungal group, we know little about how these enzymes are induced and formed in solid conditions using plant-derived residues. In our previous work, β-glucosidase activity of several zygomycetes grown in liquid and solid media was measured and some strains showed intensive extracellular enzyme activity on wheat bran (TAKÓ ET AL., 2010). This study presents the β-glucosidase production of zygomycetes strains using oilseed residues such as hempen-, line-, poppy- and pumpkin-seed and oat bran in solid-state fermentation (SSF). Five strains of the genera *Rhizomucor*, *Rhizopus*, *Mucor*, *Umbelopsis* and *Mortierella* were tested and the effect of mineral salts and olive oil on the product yield was also assessed. To our best knowledge, *Umbelopsis* and *Mortierella* isolates have never been investigated in this regard.

MATERIAL AND METHOD

Fungal strains and media for fermentation

Rhizomucor miehei (SZMC 11005; SZMC - Szeged Microbiological Collection, Szeged, Hungary), *Rhizopus stolonifer* (SZMC 13609), *Mucor corticolus* (SZMC 12031), *Mortierella echinosphaera* (SZMC 11251) and *Umbelopsis autotrophica* (SZMC 11276) strains were used for SSF. The hempen-, line-, poppy- and pumpkin seed residue substrates were remained after extraction of the plant-seed oils (Solio Ltd.). Crude fiber content of them was determined by the producer. The oat bran was purchased in a local market (Natura Ltd.).

Culture conditions for β-glucosidase production

To investigate the production of β-glucosidases on the plant residues and under different conditions, five grams of substrate were taken in 100-ml Erlenmeyer flasks and moistened with 5 ml distilled water (medium 1) or 9.5 ml mineral salt medium (medium 2; % in w/w substrate in distilled water: 0.75% $(NH_4)_2SO_4$, 0.34% NH_2CONH_2, 1.8% NaH_2PO_4, 0.3% KH_2PO_4, 0.045% $MgSO_4$ x $7H_2O$, 0.0375% $CaCl_2$). The culturing media were autoclaved, and the flasks were inoculated with a spore suspension containing 10^6 sporangiospores/ml. Cultures were grown for 7 days at desired temperatures depending on the culturing requirements of the tested strain (20 °C: *U. autotrophica*, 25 °C: *M. corticolus*, *Rh. stolonifer*, *M. echinosphaera*, 37 °C: *R. miehei*). All fermentation tests were carried out in three independent experiments.

Extraction and determination of β-glucosidase activity

Preparation of crude samples was carried out by an extraction with 30 ml of 0.1 M sodium acetate buffer (pH 6.0) at 4 °C for 24 h. The extracts were filtrated and then centrifuged at 16.200*g* for 15 min. The resulted clear supernatants were designated as crude extracts and used for enzyme activity assay. The β-glucosidase activity was determined by using *p*-nitrophenyl-β-D-glucopyranoside (*p*NPG; Sigma) as substrate in a reaction mixture containing 180 µl diluted enzyme solution and 20 µl of 7 mM *p*NPG. Reaction mixtures were incubated at 50 °C for 30 min and the reaction was stopped by adding 50 µl of 10% (w/v%) Na_2CO_3. *Para*-nitrophenol release was monitored at 405 nm in 96-well microtiter plates using an ASYS Jupiter HD microplate reader (ASYS Hitech). One unit of β-glucosidase activity (U) was defined as the amount of enzyme that released 1 µmol of *p*-nitrophenol per min under assay conditions. Enzyme activity values were expressed in U/g dry weight substrate (U/gds).

Statistical analysis

Standard deviations of means were performed with Microsoft Excel software from the Microsoft Office package. Enzyme activity values are averages counted from three independent measures.

RESULTS

Solid-state fermentation is a process when growth/cultivation of microorganisms is performed on water insoluble or sparingly water-soluble materials. Since solid medium could simulate the natural habitat of filamentous fungi, this fermentation technique is frequently used to produce inducible enzymes (MITCHELL and BEROVIČ, 2006). In this study, β-glucosidase production of five filamentous fungal strains was investigated in solid cultures using various oilseed residues and oat bran. The *R. miehei* and *M. corticolus* strains were selected on the basis of our previous screening researches (TAKÓ, 2011). The *Rh. stolonifer*, *M. echinosphaera* and *U. autotrophica* isolates showed intensive growth on these substrates in a recent assay (KOTOGÁN ET AL., 2013).

Detection of β-glucosidase activity on oilseed residues

Hempen-, line-, poppy- and pumpkin seed residues were used as substrates and moistened with distilled water (medium 1) or mineral salt solution (medium 2). The results in *Table 1* provide a summary of the β-glucosidase production obtained for each substrate and isolate. The tested isolates exhibited various enzyme yields during the fermentation tests; however, results showed that these plant residues are potential substrates for β-glucosidase production by the investigated fungi. The *R. miehei* and *M. corticolus* isolates proved to be the best producers on these substrates presenting from 1.32 to 8.16 and 0.99 to 4.75 U/gds of β-glucosidase, respectively. In general, enzyme yields were positively influenced by the addition of mineral salt solution as support; especially, in case of the *M. corticolus* isolate, at which the product yield has been enhanced for each substrate. At least 1.15 fold increases in the β-glucosidase activity could be detected by this strain.

Table 1. β-Glucosidase yield of the tested zygomycetes fungi on oilseed residues moisturized with distilled water (medium 1) or mineral salt solution (medium 2)

Substrate	β-Glucosidase activity (U/g dry substrate) [a]				
	Rhizomucor miehei	*Rhizopus stolonifer*	*Mucor corticolus*	*Umbelopsis autotrophica*	*Mortierella echinosphaera*
Hempen seed					
medium 1	4.19 ± 0.15 [b]	0.038 ± 0.003	1.29 ± 0.08	0.56 ± 0.01	0.013 ± 0.001
medium 2	4.58 ± 0.32	0.050 ± 0.003	4.75 ± 0.16	0.95 ± 0.03	0.017 ± 0.001
Poppy seed					
medium 1	6.90 ± 0.29	0.009 ± 0.001	0.99 ± 0.08	0.26 ± 0.01	0.005 ± 0.001
medium 2	1.32 ± 0.11	0.021 ± 0.001	1.15 ± 0.02	0.57 ± 0.03	0.004 ± 0.001
Line seed					
medium 1	2.50 ± 0.21	0.056 ± 0.007	2.11 ± 0.11	1.16 ± 0.06	0.034 ± 0.003
medium 2	4.69 ± 0.27	0.047 ± 0.006	2.69 ± 0.15	0.88 ± 0.06	0.033 ± 0.001
Pumpkin seed					
medium 1	3.72 ± 0.23	0.036 ± 0.002	1.10 ± 0.04	0.87 ± 0.09	0.041 ± 0.002
medium 2	8.16 ± 0.79	0.069 ± 0.001	1.51 ± 0.08	1.18 ± 0.03	0.032 ± 0.003

[a] Activities presented were measured at the 7th day of the fermentation.
[b] Values are averages calculated from the data of three independent measurements ± standard deviation.

Interestingly, there was no general correlation between the β-glucosidase activities developed on each substrate and the crude fibre content of the seeds (*Table 2*). Except in the case of *M. corticolus*, maximum β-glucosidase production could be recorded on pumpkin seed residue, which contains the lowest amount of crude fibre (3-3.5%) among the oilseed materials. One possible explanation for this phenomenon may be that the crude fibre content of this substrate is more accessible for the exoglucanases and endoglucanases produced by the fungi. To avoid the end-product inhibition effect of cellobiose (MURPHY ET AL, 2013), higher yield of β-glucosidase is required. Additionally, since certain carbohydrates (e.g. glucose, cellobiose) have strong inhibitory effect on β-glucosidases (EYZAGUIRRE ET AL., 2005), enzyme activity may also be influenced by the sugar content of the plant-seed substrates. The producer indicated up to 5 and 5.5% sugar and starch content in case of the hempen seed and poppy seed residues, respectively. The slight lower enzyme activity by *Rh. stolonifer*, *U. autotrophica* and *M. echinosphaera* on these substrates may be due to the inhibitory effect of the carbohydrates presented in the crude extract. Because of moderate tolerance to glucose has been reported for *R. miehei* and *M. corticolus* β-glucosidases (KRISCH ET AL., 2010), these enzymes could exhibit considerable activity at higher sugar concentrations. Another explanation for the variable yields would be the different extractive (e.g. flavonoids, tannins, terpenes, etc.) content of the substrates which may cause, for example, microbial growth inhibition and fibre hygroscopicity reduction (ANG ET AL., 2013); however, chemical composition analysis of the oilseed residues has not been performed.

Table 2. Crude fibre content of the oilseed residues (as given by the producer)

Oilseed residue	Crude fibre content (w/w %)
Hempen seed	13.2
Poppy seed	10
Line seed	9-10
Pumpkin seed	3-3.5

Detection of β-glucosidase activity on oat bran

While the enzyme activity of the other strains obtained on oat bran was comparable to that presented on oilseed residues, *R. miehei* was outstanding in its β-glucosidase activity using this substrate (*Table 3*). The maximum 30.39 U/gds β-glucosidase activity recorded in this research was 2.5 and 6.7 fold higher compared to the enzymes produced by *Aspergillus fumigatus* on wheat straw (SHERIEF ET AL., 2010) and *A. fumigatus* SK1 on oil palm trunk (ANG ET AL., 2013), respectively. However, the presented enzyme activity is less significant as compared with our previous study in which 229.8 U/gds product yield could be reached for the same isolate using wheat bran based SSF (TAKÓ ET AL., 2010).

Table 3. β-Glucosidase yield of the tested zygomycetes fungi on oat bran moisturized with distilled water (medium 1) or mineral salt solution (medium 2)

	β-Glucosidase activity (U/g dry substrate) [a]				
	Rhizomucor miehei	*Rhizopus stolonifer*	*Mucor corticolus*	*Umbelopsis autotrophica*	*Mortierella echinosphaera*
medium 1	26.04 ± 0.52 [b]	0.048 ± 0.002	1.19 ± 0.05	1.85 ± 0.03	0.038 ± 0.001
medium 2	30.39 ± 0.55	0.068 ± 0.001	1.69 ± 0.04	3.35 ± 0.11	0.027 ± 0.001

[a] Activities presented were measured at the 7th day of the fermentation.
[b] Values are averages calculated from the data of three independent measurements ± standard deviation.

Using oat bran as substrate, *U. autotrophica* was able to produce β-glucosidase with higher yield compared to other research on oilseed residues (see *Table 1* and *Table 3*). The exhibited 3.35 U/gds enzyme activity is significant since it was 3 fold higher than to that presented by *M. corticolus* which had been identified as strong β-glucosidase producer in our previous examinations on wheat bran (TAKÓ, 2011). It is worth to mention that there have been no previous studies on *Umbelopsis* species for their β-glucosidase activity. Activity data presented here suggest that it would be advantageous to screen these fungi for β-glucosidase production in future researches.

CONCLUSIONS

Successful production of industrially important microbial enzymes on agro- and food-industrial residues is a promising and eco-friendly approach to utilize these materials. Here, we studied the feasibility of some oilseed residues and oat bran to produce high level β-glucosidases through solid-state fermentation using zygomycetes. The investigated five fungal strains grow well during the cultivation, and they can be used for efficient utilization of these plant residues for production of extracellular β-glucosidases. In case of most fungi, pumpkin seed proved to be the best substrate for enzyme production; maximum enzyme activity was achieved by *R. miehei*. It can be concluded that the β-glucosidase yield generally increased by moisturizing of the substrate with mineral salt solution. This study also highlighted the potential of *U. autotrophica* for β-glucosidase production.

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QUALITY ASSESSMENT OF FREEZE- AND CONVECTIVE DRIED PLUM VARIETIES

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ABSTRACT

This paper deals with a comparison between two different drying processes. Hot-air and a vacuum-freeze drying processes were used for drying samples of plum (*Prunus domestica* L.) varieties (*'Cacanska lepotica'*, *'Cacanska rana'* and *'President'*). The main objective of our research was to analyse and simulate the change of the quality parameters formed during dehydration by comparing two known drying procedures convective dehydration and lyophilisation.

The study shows the drying kinetics of the two drying methods, and examines some special parameters which characterize the quality of dried products, including the microstructure, rehydration, and texture. The shape of cell structure was determined by electro-microscope. Thin layer drying model (third-degree polynomial) were applied for prediction of the freeze drying process. For model evaluation R^2 (coefficient of determination) was calculated. The model estimates showed a good agreement with experimental data. The highest values of hardness and rehydration were found freeze dried plum varieties.

Keywords: plum, drying, microstructure, rehydration, firmness

INTRODUCTION

Drying is one of the possible ways of processing vegetables and fruits. The most frequently applied method of this ancient preservation procedure is the artificial convective drying. This procedure became popular mainly as a result of its simple use and low operational costs; however, we should not forget its disadvantages, which are related to the quality of the dried product. These disadvantages include significant decreases in nutritional value, shrinkage, formation of a hard, non-permeable layer, and denaturation of proteins (BOURAOUT ET AL., 1994; LEWICKI, 1998).

Research has been conducted for a long time on preserving fruits and vegetables in such a way that they keep their original properties for the cold winter months as well. Nowadays, in the 21st century the requirements set out for dried fruits and vegetables including that they should be microbally stable, keep their physical, chemical and mechanical parameters and have excellent storage, packaging and transportation properties.

In addition, they should have high nutrient contents suitable for producing functional foods and food supplements. Only a few drying methods are suitable for satisfying the above-mentioned demands on preservation. According to our present knowledge, the most tolerant dehydrating method is vacuum freeze-drying. Better quality of lyophilized products results from the fact that the temperatures applied during lyophilization are much lower than during traditional drying and that the denaturation processes typical of the traditionally dried products does not occur. During lyophilization, no internal diffusion takes place because the sublimation starting from the surface gradually spreads to deeper layers; the ice directly passes into steam (KARATHANOS ET AL., 1996).

In this study the effect of two drying methods on drying characteristics, rehydration rate, hardness and microstructure were investigated. Moreover it was compared quality of convective dried and freeze dried plum varieties, which ones spring from Hungary. However, the study of plum drying is scarce in the literature.

MATERIAL AND METHOD

Materials

During the measurements were tested plum varieties (*Prunus domestica* L.) ('*Cacanska rana*', '*Cacanska lepotica*', '*President*') of exactly known origin purchased from local producers and traders (Nyíregyháza, Hungary).

We cleaned the material to be dried and cut it to size then placed it on the tray of the dryer in single layer. The samples were cut into 20 mm pieces, and total mass of the samples were 300 grams. We performed the drying test of the varieties both simultaneously and separately. The analyses were replicated three times.

Drying experiments

We performed the dehydration of the horticultural products (plum varieties) used in the experiments with the following dryers:

1. Convective drying - LP 302 (Labor MIM, Budapest) laboratory cylindrical drying cabinet (drying parameters: 8 h; 80 °C; 1.1-1.5 m/s).
2. Lyophilisation – Armfield FT 33 (Armfield Ltd., UK) laboratory vacuum freeze drier (drying temperature from -50 to 20°C; the pressure ranged from 80 to 100 Pa, drying time: 24-25 h).

In order to exactly analyse the processes taking place during the drying, we equipped the laboratory freeze dryer with a data recording system (platform cell – scale instrument – DATPump software).

Description of measuring instruments

The characteristics influencing the quality of the dried products were measured and evaluated with the following instruments and methods:

1. *Moisture content* measurement: PRECISA HA 60 (Precisa Gravimetrics AG, Switzerland) type quick moisture meter. The initial moisture content of the samples were found as 82.7-79.3%, (wet basis), respectively.

2. Measurement of the *drying parameters* of the convective method: TESTO 4510 type (Testo AG., Germany) measuring instrument.

3. *Structures of tissue* were examined using an electro-microscope (Bresser Biolux Al, 20x - 1280x, Bresser AG, Germany).

4. Measurement of the *rehydration activity* of the dried material in moistening agent (LIN ET AL., 1998).

The process of the experiment was as follows: we measured the weight of the samples dehydrated by various methods, then placed them in pots filled with water of 75 °C. During the experiment, we ensured the permanent temperature of the liquid by means of liquid supply. We removed the samples from the liquid after 60 min periods and eliminated the surplus moisture from their surfaces with an absorbent. At the end of the experiment we measured the weights of the rehydrated samples and calculated the rehydration rate (RR). The value of the rehydration rate (RR) shows how much the amount of the water absorbed again can increase the weight of the dried product.

The rehydration rate (RR) can be calculated in the following way (1):

$$RR = \frac{m_{rh}}{m_d},$$

where:

m_{rh} – mass of the rehydrated material [g],

m_d – mass of the dried material [g].

5. Determination of the *product hardness*: Brookfield CT3-4500 type (Brookfield Engineering Laboratories, Middleboro, USA) texture analyser. The description of the measurement process: ANTAL ET AL. (2013).

Data analysis

All data were analyzed using the analysis of variance (ANOVA). The Duncan's test was used to establish the multiple comparisons of mean values. A statistical program PASW Statistics 18 was used to perform all statistical calculations. All tests were performed in triplicate and the average values were reported.

RESULTS

Drying kinetics

During the drying process one of the most important tasks was to determine the drying curve (change of water content in function of the time).

Figure 1 demonstrates the change of moisture level in plum samples (*'President'*, *'C. Rana'* and *'C. Lepotica'*) during freeze drying and the curves fitting.

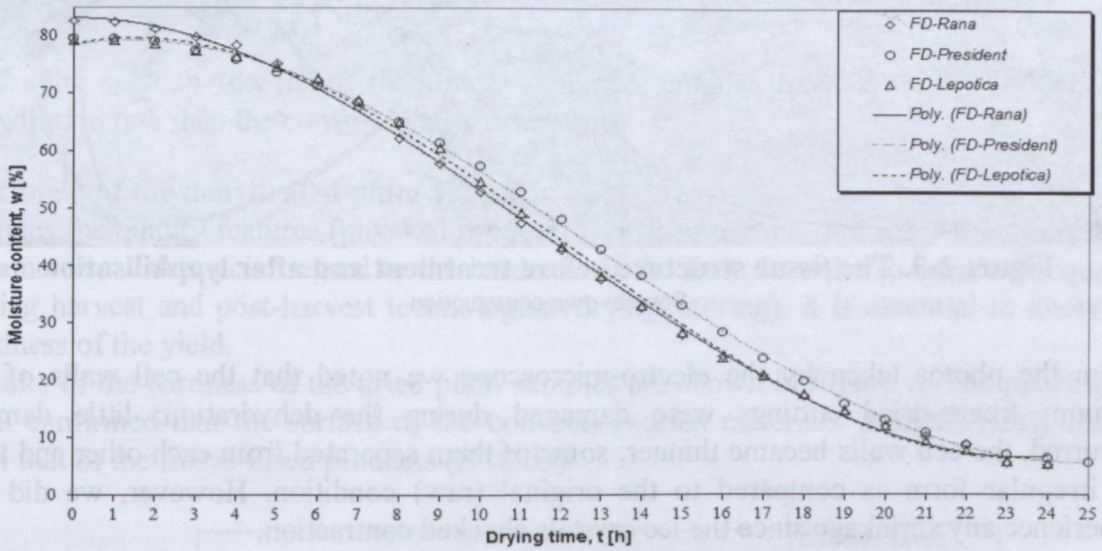

Figure 1. Drying curve of convective- and freeze dried plum slices

Source: own construction

The figure indicates that the drying period of the vacuum-freeze drying process is longer than the convective dehydration, because of the minor drying rate. The freeze drying process of plum varieties took 24-25 h, this is in agreement with YURDUGÜL AND BOZOGLU (2009). The authors reported that the wild plum duration of lyophilisation procedure 24 h.

We defined a relationship for the characterisation of the drying processes of lyophilised plums. The processes can be approximated with third-degree polynomials. The functions representing the moisture content reduction of the drying materials can be described with the following equation: $w=at^3+bt^2+ct+k$, where: w – moisture content of the product [%]; t – drying period [h], a, b, c, k – drying constants of the third-degree polynomial the values of which depend on the characteristics of the material: the variety, the freezing speed, the ripeness and the tendency to lose water.

The drying curve of the lyophilisation describes by a higher degree polynomials; the drying constants and statistical evaluation can be read in the *Table 1.*

Table 1. Drying constant of the third-degree polynomial and statistical evaluation

Plum varieties	Drying constant				Statistics
	a [-]	b [-]	c [-]	k [-]	R^2
Cacanska rana (d.t.=24 h)*	0,0121	-0,4266	0,0658	83,15	0,9990
Cacanska lepotica (d.t.=24 h)*	0,0144	-0,5306	1,4787	78,553	0,9987
President (d.t.=25 h)*	0,0117	-0,4565	1,217	78,393	0,9995

* drying time
Source: own construction

Microstructure of plum after treatments

We describe the deformations and damage of the plant tissues under the effect of the drying with microscopic tests. A range of 10 times magnification was used in the images. *Figure 2* shows the tissue of the '*Cacanska rana*' variety plum at the beginning of the dehydration, while *Figures 3* and *4* show the lyophilized and convective dried *Rana* plum samples under the microscope.

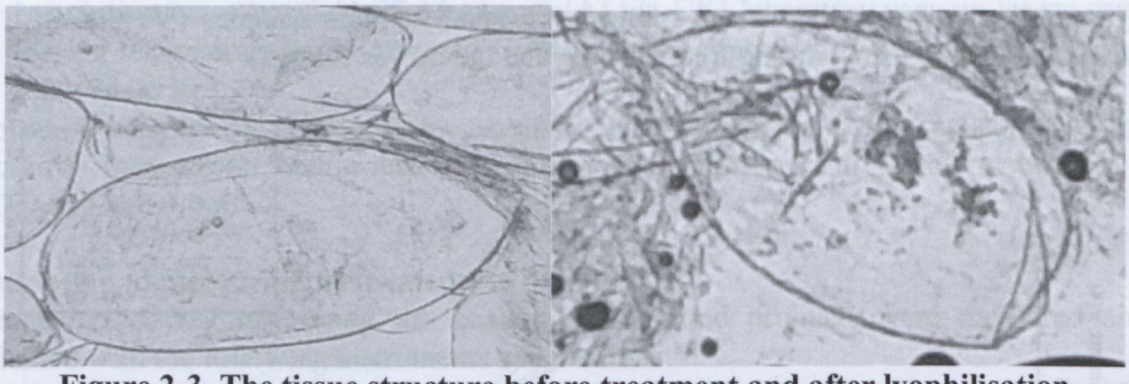

Figure 2-3. The tissue structure before treatment and after lyophilisation
Source: own construction

From the photos taken by the electro-microscope we noted that the cell walls of the vacuum freeze-dried cuttings were damaged during the dehydration; little damage occurred, the cell walls became thinner, some of them separated from each other and took an irregular form as compared to the original (raw) condition. However, we did not experience any shrinkage since the ice crystals checked contraction.

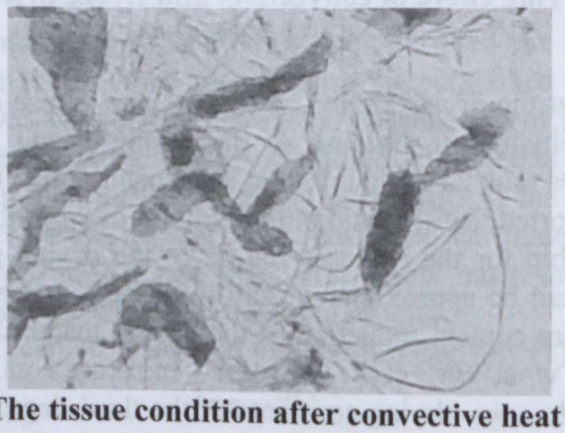

Figure 4. The tissue condition after convective heat treatment
Source: own construction

For the samples dried with the traditional method, the cellules shrunk, the cell walls became thinner, separated from each other and went through deformation which did not recover even during rehydration.

Effect of various drying methods on the rehydration

The rehydration curves of the dried plum samples are illustrated in *Figure 5*, at 75 °C water temperature. We indicated the significant differences of the treatments on the figure (*P<0.05*).

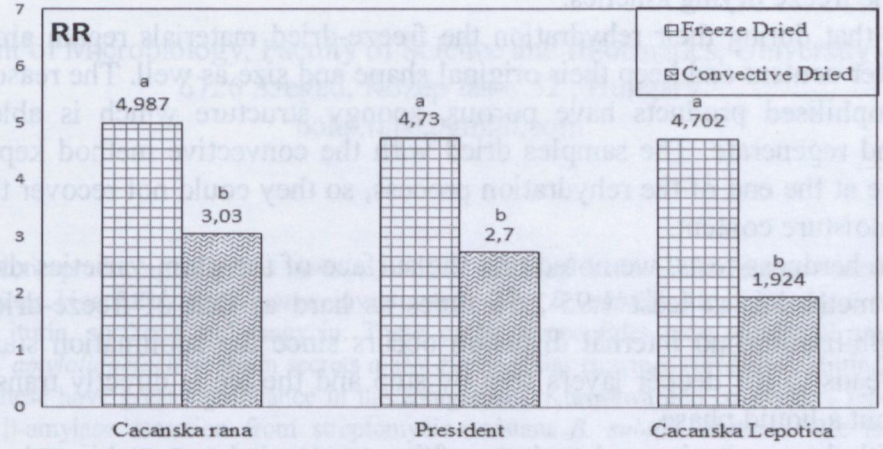

Figure 5. Rehydration capacity of the dried plum varieties

Source: own construction

Different letters in the same column indicate a significant difference (p<0.05), Duncan test

It can be seen in the figure that the lyophilized samples have a significantly higher rehydration rate than the conventionally dried fruits.

Hardness of the dehydrated plum varieties

Besides the quality features (physical properties), it is important to discuss the mechanical parameters, with special regard to the hardness of the fruit. In order to ensure the quality during harvest and post-harvest technologies (drying, storing), it is essential to know the hardness of the yield.

Results of the hardness of the dried plum samples are shown in *Figure 6.* The penetration tests confirmed that the surface of the convection-dried materials is significantly harder than that of the freeze-dried products (*P<0.05*).

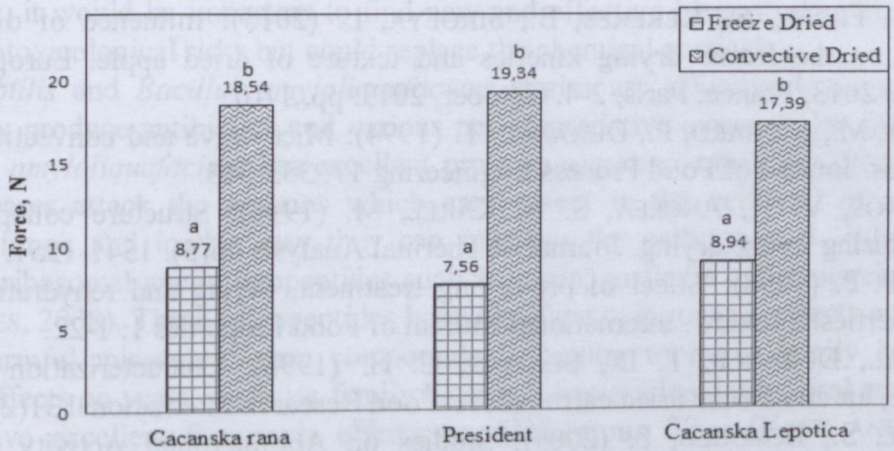

Figure 6. Comparison of the surface hardness of dried plums

Source: own construction

Different letters in the same column indicate a significant difference (p<0.05), Duncan test

CONCLUSIONS

With regard to the results of the drying kinetics, we found that the temperature and pressure applied for the freeze-drying is much less while the drying time is much longer than those for the convection drying. The third-degree polynomial model adequately described the freeze drying kinetics.

We proved that during their rehydration the freeze-dried materials regain almost at their original water content and keep their original shape and size as well. The reason for this is that the lyophilised products have porous, spongy structure which is able to absorb moisture and regenerate. The samples dried with the convective method kept their hard, solid surface at the end of the rehydration process, so they could not recover their original shape and moisture content.

Through the hardness tests, we noted that the surface of the plum varieties dried with the convection method is at least 1.95-2.56 times as hard as that of freeze-dried products. During lyophilisation, no internal diffusion occurs since the sublimation starts from the surface, spreads to the deeper layers step by step and the ice is directly transformed into steam without a liquid phase.

We dealt with the monitoring and analyses of the structural changes taking place during the drying process. The specimens were analysed and recorded by means of a transmission electro-microscope. From the photos taken we revealed that for the lyophilised products the tissue became less damaged and the cell walls were less deformed by the drying process than for the materials dried with the convection method.

ACKNOWLEDGEMENTS

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CHARACTERISATION OF STREPTOMYCIN RESISTANT MUTANTS OF BIOCONTROL *BACILLUS* STRAINS

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ABSTRACT

A good antibiotic-producing *Bacillus subtilis* and an elevated extracellular enzyme-secreting *Bacillus amyloliquefaciens* biocontrol strain were investigated. The *B. subtilis* strain produces non-ribosomal oligopeptides: iturin, surfactin and fengycin. These cyclic lipopeptides have antifungal and antibacterial effects. The *B. amyloliquefaciens* strain secrets many extracellular enzymes (proteases, chitinases, cellulases and lipases); these have great significance in the antagonism. Kurosawa and co-workers reported elevated protease and β-amylase secretion from streptomycin-resistant *B. subtilis* mutants. We isolated twenty spontaneous streptomycin resistant mutants from our *B. subtilis* strain and four spontaneous streptomycin resistant mutants from the *B. amyloliquefaciens* strain. We investigated the extracellular enzyme and antibiotic production of the mutants. Some of the mutants showed elevated enzyme and antibiotic secretion. The *rpsL* gene in theses spontaneous streptomycin resistant mutants, were sequenced and point mutations were detected in it, so very likely the changes of the structure of the rpsL protein is responsible in some cases for the elevated enzyme and depsipeptide production.

Keywords: antibiotic, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, biocontrol, extracellular enzymes

INTRODUCTION

The proper control of the different pests (e.g. plant pathogenic fungi) is a basic requirement in the modern agriculture. Most of the common agricultural technologies rely on the extensive use of pesticides; this greatly contributes to the increased growth yields and the production of quality of food and feeds. At the same time, the widespread use of chemical pesticides considerably increased the environmental problems of the agricultural areas. Accordingly, it would be important to find new and effective biocontrol agents which do not have ecotoxicological risks but could replace the chemical pesticides.

Bacillus subtilis and *Bacillus amyloliquefaciens* strains are effective biocontrol agents, because they produce antibiotics and various pest-degradative extracellular enzymes. For example, *B. amyloliquefaciens* has excellent protease secreting capability (PRIEST, 1977). These proteases attack the proteins which are present in the cell-wall of many plant pathogenic fungi, and in this way they can suppress the pathogens. *B. subtilis* strains secrete non-ribosomal cyclic lipopeptides such as iturin, surfactin and fengycin (ONGENA AND JACQUES, 2008). These oligopeptides have excellent antagonistic effects against wide range of harmful microorganisms: compounds belonging to iturin family have strong antifungal effects on yeast, surfactin family have anti-bacterial and anti-viral activities and fengycin have excellent fungitoxic effects on filamentous fungi (KATZ AND DEMAIN, 1977).

KUROSAWA and co-workers (2006) reported the elevated protease and α-amylase secretion of streptomycin resistant *B. subtilis* strains. This phenomenon appeared in connection with spontaneous point mutations in the *rpsL* gene. This gene encodes the protein S12 of the 30S ribosomal subunit. In this work we report the successful isolation of a potent antagonistic *B. subtilis* and *B. amyloliquefaciens* strains from the rhizosphere of tomato

plants and the isolation and characterization of spontaneous streptomycin resistant mutants of these strains.

MATERIAL AND METHOD

Isolation of spontaneous streptomycin resistant *B. subtilis* and *B. amyloliquefaciens* strains

Bacillus cells from a 24 h liquid culture were suspended in 0.5 ml of 1% NaCl solution (5 x 10^7 cell ml^{-1}). Fifty µl of this suspension were spread on the surface of YEG medium supplemented with 100 µg ml^{-1} streptomycin. After one week the appearing colonies were picked and further subcultured (purified) on streptomycin-containing medium.

Investigation of the extracellular enzyme production of *B. subtilis* and *Bacillus amyloliquefaciens* strains

Medium for enzyme production was used as reported by BESSON ET AL. (1987): (constituents in g l^{-1}): glucose 10, glutamic acid 5, KH_2PO_4 1, K_2HPO_4 1, $MgSO_4$ x $7H_2O$ 0.5, KCl 1, $FeSO_4$ x 7 H_2O 0.005, $CuSO_4$ x $5H_2O$ 0.00016. Non-inductive enzyme production was measured after 7 days with chromogenic protease and chitinase substrates. Bz-Phe-Val-Arg-pNA, Suc-Ala-Ala-Pro-Phe-pNA and 4-nitrophenyl-N-acetyl-glucosaminide were used for trypsin-type protease, chymotrypsin-type protease and for exochitinase measurements, respectively.

Investigation of the antibiotics producing capabilities

The medium used for antibiotic production was reported by BESSON ET AL. (1987). The antibiotics were precipitated from the ferment broth by lowering the pH to 2 with HCl. The pelleted antibiotics were dissolved in ethanol and separated by thin layer chromatography (TLC) on Kieselgel 60 plates with chloroform:methanol:water (65:25:4) eluent. The separated antibiotics were visualized with a color reagent containing 3 g phenol, 94.98 ml ethanol, 5 ml sulfuric acid and 20 µl anisaldehide. The amount of the secreted, tyrosine containing antibiotics was measured spectrophotometrically at 280 nm.

Amplification of the *rpsL* gene

The *rpsL* gene from the wild-types and from the streptomycin resistant mutants were amplified by polymerase chain reaction (PCR) and sequenced. PCR was carried out in a final volume of 50 µl containing 5 µl of *Taq* polymerase 10× buffer, 1.6 mM $MgCl_2$, 200 µM for each of the dNTPs, 10 pM primers, 2 µl of template DNA in distilled water and 1 U of *Taq* DNA polymerase. The following primers were used BF-1 5' ATGCCTACAATTAATCAGCTAATTC 3' and UP BR-1 5' TACGGATGTTAATTAGTCGATTAAG 3'. Amplification was performed in a T3 thermocycler as follows: 1 cycle at 94°C for 5 min, 35 cycles at 94°C for 30 s, 50°C for 40 s, and 72°C for 1 min and a final elongation step at 72°C for 3 min. PCR products were separated by electrophoresis (1.5% agarose gel prepared in TAE buffer containing ethidium bromide) and investigated under UV light.

RESULTS AND DISCUSSION

Isolation of streptomycin-resistant *B. subtilis and B. amyloliquefaciens* strains

Twenty spontaneous streptomycin resistant *B. subtilis* mutants were isolated. Ten of them belonged to morphotype-1; these were characterised with about 1 mm colony diameter with regular colony edge. Ten of them belonged to morphotype-2; these were characterised with 2-4 mm colony diameter with irregular edges.
From the *B. amyloliquefaciens* strain four spontaneous streptomycin-resistant mutants were isolated. All of these mutants showed morphotype-1 character.

Investigation of the extracellular enzyme production of *B. subtilis* and *B. amyloliquefaciens* strains

Trypsin-type protease, chymotrypsin-type protease and exochitinase activities in the ferment broths of wild-type and of the streptomycin-resistant mutants were measured. Two of the streptomycin-resistant mutants of *B. subtilis* had increased trypsin-type protease and three had increased chymotrypsin-type protease activities (*Figure 1* and *Figure 2*). The K2 mutant had nearly fourfold increase in chymotrypsin activity. We did not detect elevated exochitinase activity of the streptomycin resistant mutants.

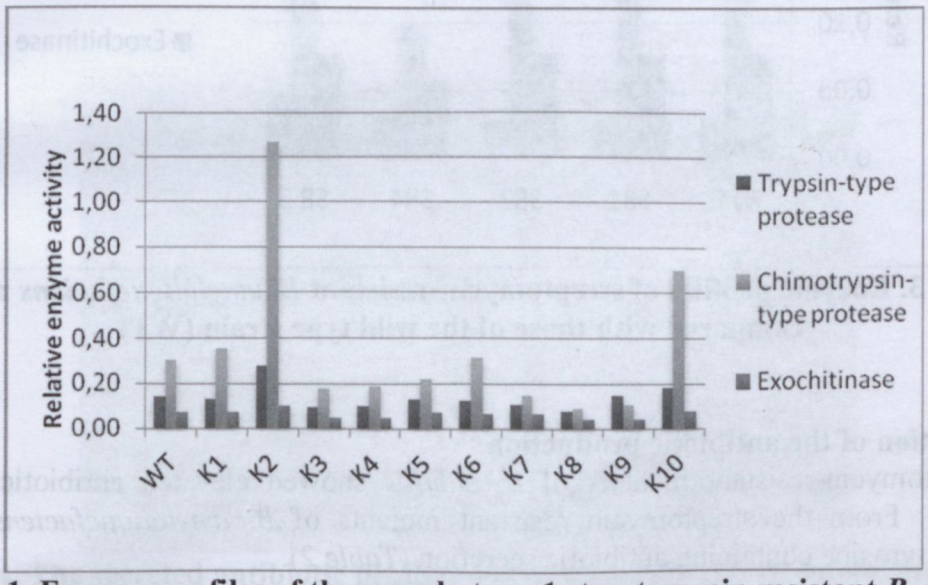

Figure 1. Enzyme profiles of the morphotype-1 streptomycin resistant *B. subtilis* mutants compared with those of the wild type strain (WT)

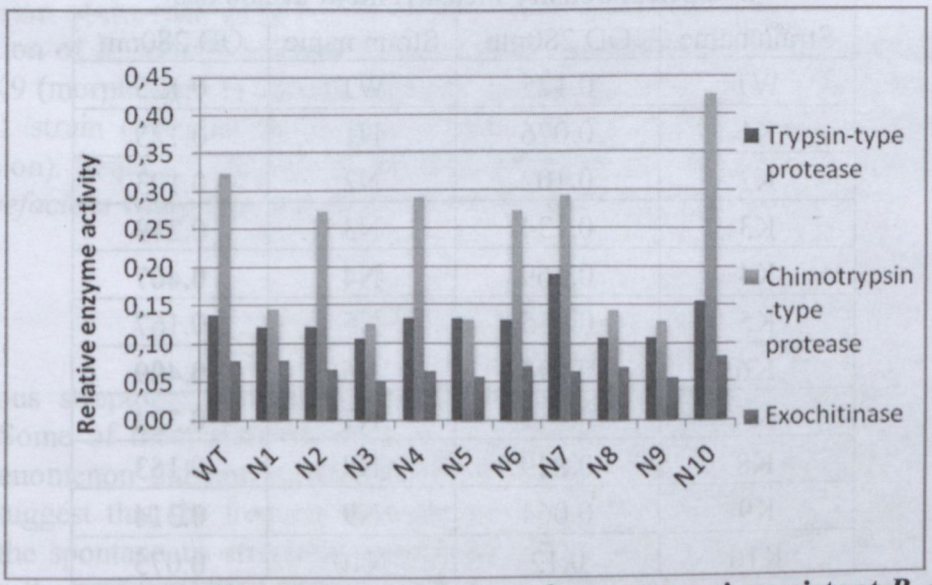

Figure 2. Enzyme profiles of the morphotype-2 streptomycin-resistant *B. subtilis* mutants compared with those of the wild type strain (WT)

The mutant *B. amyloliquefaciens* strain *SR5* had elevated chymotrypsin-type protease activity compared with the wild type (*Figure 3*).

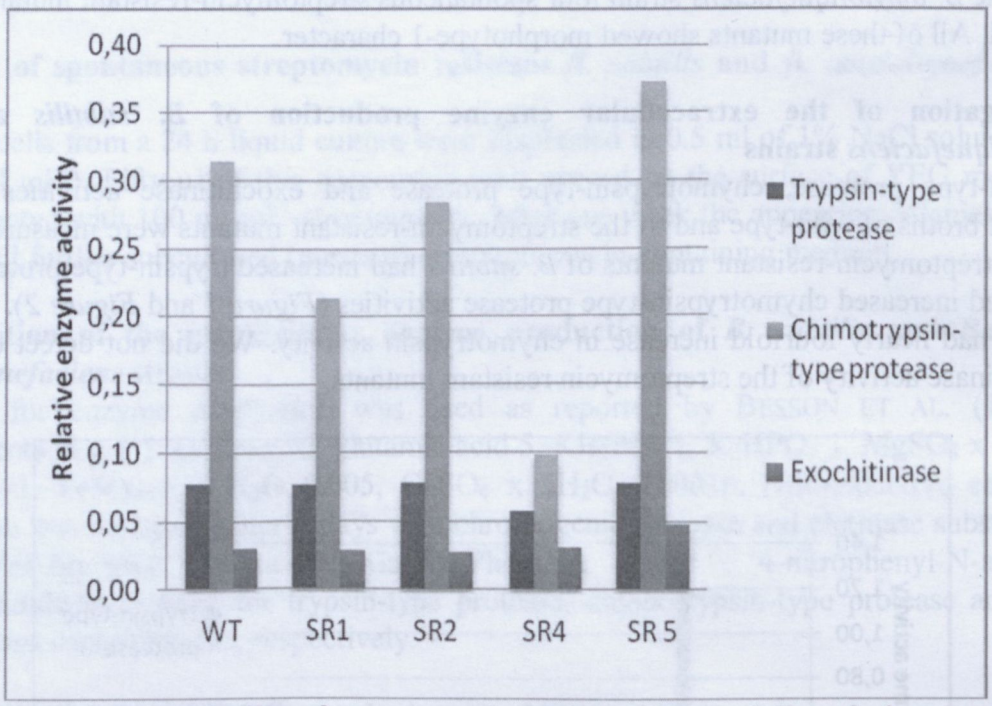

Figure 3. Enzyme profiles of streptomycin-resistant *B. amyloliquefaciens* mutants compared with those of the wild type strain (WT)

Investigation of the antibiotic production

Six streptomycin-resistant mutants of *B. subtilis* showed elevated antibiotic secretion (*Table 1*). From the streptomycin resistant mutants of *B. amyloliquefaciens* revealed increased tyrosine containing antibiotic secretion (*Table 2*).

Table 1. *B. subtilis* tyrosine-containing antibiotics in the ferment broths determined by optical density measurement at 280 nm

Strain name	OD 280nm	Strain name	OD 280nm
WT	0.125	WT	0.125
K1	0.076	N1	0.125
K2	0.102	N2	0.193
K3	0.134	N3	0.219
K4	0.169	**N4**	**0.467**
K5	0.035	N5	0.167
K6	**0.495**	**N6**	**0.409**
K7	0.051	**N7**	**0.324**
K8	**0.829**	N8	0.153
K9	0.064	**N9**	**0.314**
K10	0.12	N10	0.075

Table 2. *B. amyloliquefaciens* tyrosine-containing antibiotics in the ferment broths evaluated by optical density measurement at 280 nm

Strain name	OD 280nm
WT	0.337
SR1	0.319
SR2	0.272
SR4	**0.707**
SR5	0.361

The secreted antibiotics were visualized by TLC (*Figure 4*). *B. subtilis* strains showed outstanding surfactin producing abilities. This surfactin production slightly varied in the mutants. Surprisingly, the iturin production disappeared in the streptomycin-resistant strains. Fengycin production was very high in *B. amyloliquefaciens* strains. As in the case of SR4 mutants the fengycin disappeared from the ferment broth, the measured high optical density at 280 nm could origin from secreted free tyrosine or tyrosine containing proteins.

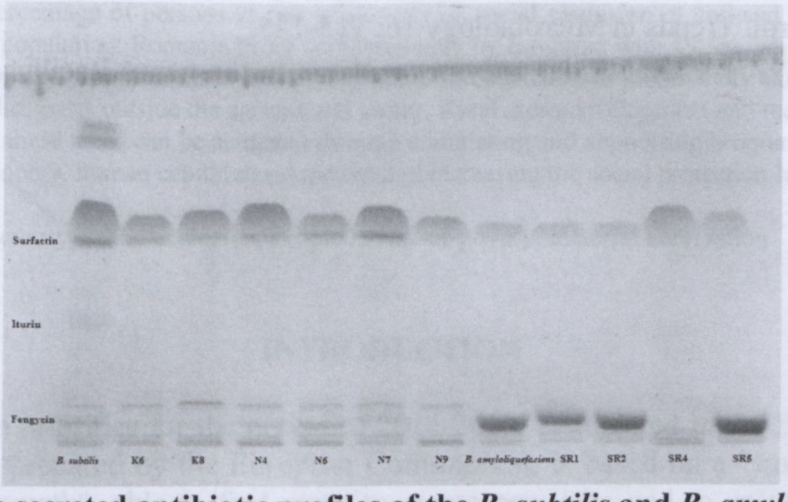

Figure 4. The secreted antibiotic profiles of the *B. subtilis* and *B. amyloliquefaciens* strains

Investigation of the *rpsL* gene

Investigation of *B. subtilis* mutants revealed point mutations in the sequence of the *rpsL* gene: in K9 (morphotype-1) adenine changed to guanine in the nucleotide 214 (transition). In the N1 strain (morphotype-2) guanine changed to thymine in the position of 103 (transversion). Sequence analysis of the *rpsL* gene did not reveal differences between *B. amyloliquefaciens* wilde-type and its streptomycin-resistant mutants strains.

CONCLUSIONS

Spontaneous streptomycin-resistant *B. subtilis and B. amyloliquefaciens* strains were isolated. Some of them showed elevated extracellular enzyme and antibiotic secretion. These phenomenon did not correlated with sequence changes in the *rpsL* gene. These findings suggest that the frequent abundance of strains with elevated secretion abilities amongst the spontaneous streptomycin-resistant mutants is not always related to genetic changes in the *rpsL* gene, but the method is useful for the breeding of biocontrol *Bacillus* strains.

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RESEARCH ON RURAL POVERTY IN ROMANIA

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ABSTRACT

One of the European Union's priorities for the programming period of 2014-2020 consists of inclusive favourable growth. This means promoting an economy with a high rate of employment, to ensure economic, social and territorial cohesion such that the benefits of economic growth and jobs to be distributed fairly, and people experiencing poverty and social exclusion to be given the opportunity to have a decent life and to play an active role in the society. The achievement of stated strategic objective requires a special attention on rural areas, both at EU level, as at each Member State level. In Romania, 45.0% of the country population is concentrated in rural areas, where there are living almost three quarters (71.3%) of the country's poor population. The percentage of persons at risk of poverty or social exclusion at national level in 2012 was 41.7% of the total population, Romania being surpassed only by Bulgaria, with 49.3%, while the EU average stood at 24.8%. Poverty in rural areas is due mainly to the low agricultural productivity and low employment opportunities in other areas outside the agricultural sector. Rural areas development and reduction of the high level of poverty in these areas can be achieved through stimulating and supporting programs of development for rural social economy, human capital development and increasing the social protection level in rural areas.

Keywords: rural poverty, social exclusion, people at-risk-of-povery, material deprivation

INTRODUCTION

European Union priorities for the period of 2014-2020, formulated in the Europe Strategy 2020 document prepared by the European Commission, is based on a "smart, sustainable and inclusive growth", able to find ways to create new jobs and to ensure better living conditions (EC, 2010). Thus, it aims to promote social inclusion, in particular through "poverty reduction, aiming to eliminate the risk of poverty and exclusion for at least 20 million people" (EC, 2012).

Romania's objective in this regard is to reduce the number of people at risk of poverty or social exclusion by 580,000 people by 2020 (EC, Europe 2020 targets), which represents a reduction of about 15.0% compared to 2008.

MATERIAL AND METHOD

The methodological path followed in this study has two research directions. The first line of research aims at identifying the factors underlying poverty in rural areas. Of the multitude of factors determining rural poverty, education and employment play a key role. The indicators used to determine the incidence of these two factors on poverty are:

1. The size of population economically active, reflecting the size of the pool of workforce recruitment including all persons who have a job or are looking for a job.
2. Activity rate, calculated as a percentage of the active population in the working age population, measures the relative availability of working age population to be involved in economic activities.

3. The employment rate of rural population, calculated as the ratio between employed population and total population, highlights the risk of not having a job and therefore not being eligible for incomes or other benefits to meet the existential needs.
4. The unemployment rate, calculated as the share of unemployed in the active population, expressing the vulnerability to unemployment for the active population and indicates the directions that are needed to be targeted the employment programs.
5. The structure of the unemployed by level of education (the share of unemployed with the educational level x in the total number of unemployed) reflects the risk of being unemployed based on the level of education and shows which are the most vulnerable categories that require corrective interventions through training and retraining courses to increase the opportunities for applying to a job.
6. The distribution of employed population in the rural area by economy sectors, is the share of employed population in the formal sector, the informal sector and household sector.

The second methodological approach includes the presentation of indicators on poverty measurement. According to the Eurostat methodology, in order to measure poverty, there are used three indicators: 1) people at-risk-of-poverty after social transfers, 2) severely materially deprived people and 3) people living in households with very low work intensity.

1. People at-risk-of-poverty after social transfers represents the percentage of people who have an equivalised disposable income below 60.0% of the national median equivalised disposable income after social transfers.
2. Material deprivation covers indicators relating to economic strain and durables. Severely materially deprived persons live in conditions greatly constrained by a lack of resources and cannot afford at least four of the following: to pay rent or utility bills; to keep home adequately warm; to pay unexpected expenses; to eat meat, fish or a protein equivalent every second day; a week's holiday away from home; a car; a washing machine; a colour TV; or a telephone.
3. Persons living in households with very low work intensity represent the persons who are aged 0-59 and the working age members in the household worked less than 20.0% of their potential during the past year.

RESULTS

Determinant factors of poverty

The causes of rural poverty are complex and multidimensional. They involve, among other things, culture, climate, gender, markets, and public policy (MAHMOOD, 2001).

Causes of poverty in rural areas, which are most often discussed in the literature, include low income, lack of employment, the high costs of new housing construction, poor quality of housing (leading to higher costs for heating), poor health and lack of healthcare within a reasonable traveling distance, and low levels of education (BURNS, BRUCE, MARLIN, 2013).

In Romania, the analysis of poverty risk shows that the determinant factors of this phenomenon are multiples, starting from demographic determinants, of employment, of incomes and expenses, of housing, heritage and properties, of education, health, social networking, to community factors (PARASCHIV, 2008). Among these factors, the most important are education and employment, which, moreover, are strongly correlated. An educational level and of higher professional qualification provides to individuals the opportunity to have access to a work place with a certain earning and getting a better

position on the labor market and, consequently, a better income, regular and safe, which is providing protection against poverty.

In Romania, in the year 2010, the active population from the rural areas was 4.4 million of people, the volume of the working population being on a downward trend, which means that a share increasingly higher of working age population thickens among economic inactivities, being excluded from the labor market. The total employed population numbered about 4.2 million people, following the same downward trend as the active population. In the working age population (15-64 years old), the employment rate was 60.9 %, declining in recent years in the context of the global financial crisis - *Table 1.*

Table 1. Indicators of workforce in rural areas from Romania

	2007	2010
Economically active population, thou. persons	4500	4427
Employment, thou. persons	4281	4208
Unemployed, thou. persons	219	219
Activity rate, %	65.1	64.4
Employment rate, %	61.5	60.9
Unemployment rate, %	4.9	5.0

Source: NIS, Romanian statistical yearbook, 2009 and 2011

At national level, the employment rate has a lower level compared to the European average (64.3%) and is still far from the national target of 70.0% assumed in the context of Europe Strategy 2020 (*Table 2*).

Table 2. The employment rate of working age population 20-64 years old (%)

	2002	2005	2008	2009	2010	2011	*Europa 2020 targets
RO	63.3	63.6	64.4	63.5	63.3	62.8	70.0
UE-27	66.7	68.0	70.3	69.0	68.6	68.6	75.0

Source: Eurostat;* EC, Europe 2020 targets

The occupation model from Romania is very different by residence areas (urban or rural), but also to the other Member States of the European Union. The share of employees is very low, about 67.0% (compared to 80.0% and more than this in most EU countries), with significant differences between urban and rural areas. In rural areas the share of employees from the employed population is very low, only 36.0% and the proportion of self-employed (including unpaid family workers) is extremely high, 64.0% (nationally, the proportion of self-employed is 33.0-35.0% to 15.0-16.0% of EU-27 average). This structure is associated with the importance of employment in the agricultural sector, especially in rural areas where farming population is 62.0%, compared to 33.0% nationally and 5.0% in the EU-27 average (NIS, 2011).

The educational level is another important determinant of occupational vulnerability, the reduced number of schooling years may be a prerequisite for occupational eliminating. In the total volume of unemployed, the majority are those who have previous occupational experiences, representing 86.2%. People who do not receive unemployment benefits are 47.4% of the total of unemployed. In the subgroup "unemployed with work experience" the highest share is for people with primary, secondary and vocational education: 60.7%; persons with higher education showed a significant share value of 9.9%. In the subgroup of "unemployed with no work experience", the highest share represents people who have a

consistent educational capital: 58.0% had completed secondary and post-secondary studies and 28.1% people who graduated higher education. People who do not have a job and not receiving unemployment benefits are mostly those who have completed primary, secondary and vocational school: 88.4%. The weight value decreases as the number of years of education is amplified: 8.6% have attended secondary and post-secondary schools and 3.0% are graduates of university education forms (*Table 3*).

Table 3. Educational structures of unemployment, 2010 (%)

	Unemployed with work experience *	Unemployed with no work experience *	Persons who do not receive unemployment benefits
Primary, secondary, vocational school	60.7	13.9	88.4
Secondary and post-secondary schools	29.4	58.0	8.6
Universitary	9.9	28.1	3.0

* unemployment benefit recipients
Source: NIS, Romanian statistical yearbook, 2011

In Romania, the informal employment is widespread (GOVERNMENT OF ROMANIA, MLFSPE, 2013). In 2011, a study funded by Sectoral Operational Programme Human Resources Development, implemented by the National Trade Union Bloc showed that in rural areas 65.0% of the total employed populations, including those from subsistence farming, are working in the informal sector. Although the majority consists of employment in the sector of own households (51.3%), nearly 5.7 million people are working as employees under a verbal agreement, with no employment contract, or are employed in unregistered economic units. As in other countries, a substantial proportion of households combines the subsistence agriculture with informal occasional works (mostly agricultural day laborer). The informal money incomes deepen the inequality so in the informal sector the rich become richer and the poor barely survive (STĂNCULESCU, POP, 2009).
From the analysis of presented data, we can conclude that a significant part of the rural employed population has insecure jobs, seasonal or occasional ones, of which they are obtaining low and irregular incomes (often in kind) and are not covered by the social, health and unemployment insurance system, contributing to deepening of poverty in rural areas.

Poverty assessment indicators

Measuring poverty and social exclusion is difficult because it is a multidimensional concept. As household income is generally considered a key determinant of standard of living, the at-risk-of-poverty after social transfers indicator is a meaningful measure of poverty. However, other relevant barriers to full participation in society, such as access to the labour market and material deprivation, also need to be considered (EUROSTAT, 2011). The complex nature of social exclusion is one reason why the European Commission has adopted the broader "at risk-of-poverty-or-social-exclusion rate" indicator in its Europe 2020 Strategy. The at-risk-of-poverty-or-social-exclusion indicator is an aggregate of three sub-indicators important to the Europe 2020 Strategy: people at-risk-of-poverty after social transfers, severely materially deprived people and people living in households with very low work intensity. The strategy promotes social inclusion by aiming to lift at least 20 million people out of the risk of poverty and social exclusion. The indicator also plays an

important role in the Strategy's flagship initiative "European platform against poverty" to ensure social cohesion.

In Romania, in the year 2012, 12,430 thou people (41.7%) of the total population were on the brink of poverty or social exclusion, of which 4,824 thou people were at risk of poverty (with an income below 60.0% of average disposable income), 6,391 thou people were facing severe material deprivation and 1,215 thou people were living in households with very low work intensity. Poverty or social exclusion degree from Romania increased in 2012 compared to 2011, when this indicator was at 40.3%, but is decreasing compared to 2007, when poverty degree was 45.9%. This indicator places Romania on the first places in Europe, Romania being surpassed by Bulgaria only by 49.0%, while the EU average is around 25.0% (EUROSTAT).

Table 4. Indicators of poverty

Indicators	RO		EU-27	
	2007	2012	2007	2012
People at-risk-of-poverty or social exclusion, % of population	45.9	41.7	24.4	24.8
Risk of poverty after social transfers, % of population	24.8	22.6	16.5	16.9
Severely materially deprived people, % of population	36.5	29.9	9.1	9.9
Very low work intensity, % of population	8.4	7.4	9.7	10.3

Source: Eurostat, online data codes: tsdsc 100, tsdsc 280, tsdsc 270, tsdsc 310

The indicators of poverty in Romania followed a downward trend until 2011, following an increase in 2012 (*Table 4*). The economic downturn led to a discontinuation trend of reducing the risk of poverty or social exclusion both in Romania and in Bulgaria (EC, 2012).

The dominant form of poverty in Romania is severe material deprivation which affected 29.9% of the population in 2012, compared to 9.9% of EU27 average. Of the types of deprivation, prevalent is economic deprivation, which has increased starting with 2010. The lack of financial ways results in insufficient food, poor housing conditions, difficulty to deal with unexpected expenses, lack of annual holiday. The deprivation given by poor equipment with durable goods is primarily concerned of the lack of a car (NIS, 2010). European comparisons highlight the fact that Romania is significantly weaker positioned to all Member States except Bulgaria, in terms of forced absence (due to insufficient funds) of the following goods: computer and internet, eating meat or fish at least once every two days, replacing old and worn clothes with new ones, two good pairs of shoes, of which one for all seasons, and regular leisure activities (EC, EUROSTAT, 2012).

Population living in households with very low work intensity is 7.4% of the population (in 2012) compared to 10.3% EU27 average. However, about one in ten children and one in ten people aged 18-59 years are living in households where no member is an employed person (MLFSPE).

At the territorial level (*Figure 1*), significantly higher percentages of people at-risk-of-poverty or social exclusion are in rural areas and small towns, especially in the North-East, South East, South West and South- Muntenia. Over 71.0% of Romania's poor population lives in rural areas where, according to the INS, the risk of extreme poverty is four times higher compared to urban areas (8.8% vs. 2.2%).

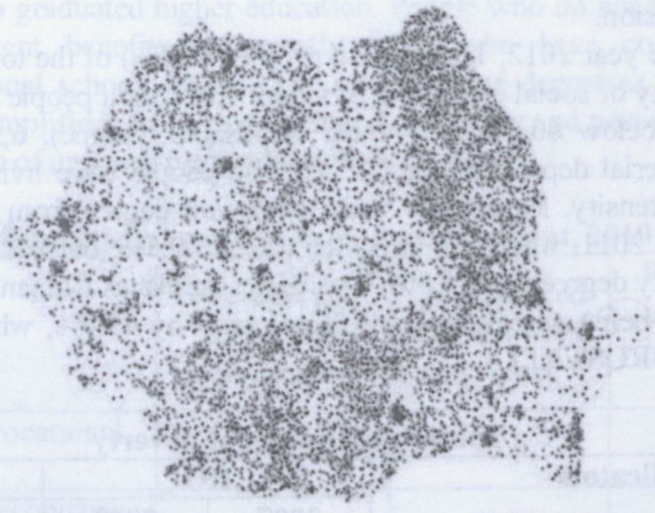

Figure 1. Distribution of poverty in Romania in 2011 (NUTS 3 level)
Each dot represents 400 people who live below the poverty
Source: Based on Census of Population and Housing 2011 and 2011 EU-SILC survey,
Retrieved on http://www.capital.ro/harta-saraciei-in-romania-190643.html

Urban areas affected by poverty include small towns experiencing population aging and depopulation, mono-industrial cities (eg. mining towns), agricultural towns or newly established cities. Small towns concentrate poverty because of poor physical infrastructure (transport, health, education), in addition, they were significantly affected by industrial restructuring and are very vulnerable to economic and industrial reform, whose main effect is a low rate of employment and therefore fragile and lower revenues.

Isolated settlements in mountainous areas or regions along the Danube corridor suffer the same negative trend as a result particularly of difficult geographical positioning and limitations concerning activities and employment. Poverty level is very high in these areas, requiring an integrated approach to meet their complex needs of development. Mapping poverty in rural and urban areas shows a lower risk of poverty in rural areas that are close to a big city. Wihin the areas where there are any urban centers or within the periphery of small or underdeveloped towns, the trend is of increasing poverty (MINISTRY OF EUROPEAN FUNDS, 2013).

The low living standard of the rural population associated with a reduced degree of growth and organization of food products market leads to a high percentage of self-consumption in total consumption of the population. In the current economic model of households in Romania, food consumption from own production and those received from extended family (parents, brothers) is one of the ways to cover the needs of food consumption, being in the same time a barometer of the economic development level (HURMUZACHE ET AL., 2013).

In order to reduce the incidence of poverty of Romanian rural, there are imposed two distinct sets of measures, one on short and medium term (horizon 2014-2020) and other on medium and long term (horizon 2020 to 2030), with specific strategic objectives of each stage. Thus, on short and medium term, the strategic objective consists in mitigating the negative effects of this phenomenon, and at the horizon of years 2020-2030 it aims attacking the root causes of the emergence and perpetuation of poverty (STERIU AND OTIMAN, 2013).

CONCLUSIONS

Europe Strategy 2020 sets out one of the priorities of the European Union for the period after 2013 as being the development of an economy with a high rate of employment, able to ensure economic, social and territorial cohesion.

A sustainable development can not be achieved without taking into account the spatial dimension of poverty. The percentage of persons at risk of poverty or social exclusion at national level in 2012 was 41.7% of the total population, Romania being surpassed only by Bulgaria, with 49.3%, while the EU average stood at 24.8%.

Poverty is significantly higher in rural areas where there are living almost three quarters (71.3%) of the country's poor population. Here, poverty and deprivation are combined in terms of housing, specifically concerning the underdevelopment of hygiene infrastructure (toilet, bathroom, running water).

In rural areas, the main problem is poverty of the traditional type, associated with the low level of modernization of the village and, in particular, of urban and social infrastructure development, namely economic life dominated by agriculture.

Rural areas development and reduction of high poverty level in these areas can be achieved through incentive programs and support for the development of social rural economy, human capital development and the increase of social protection level in rural areas.

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THE EFFECT OF ARSENIC-TREATED IRRIGATION WATER ON THE CONTENT AND DISTRIBUTION OF ARSENIC IN ROOT, LEAF AND BERRY OF TOMATO

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ABSTRACT

The horticultural, and mainly vegetable growing, is one of the most important sectors of agriculture in Hungary. The production area of indoor and outdoor vegetable growing exceeds 60 000 ha per year. About 80 percent of this area is located in the southeast region of the country. The average precipitation of this area is 380-450 mm per year, therefore intensive vegetable growing can't avoid irrigation. However, sprinkling water is relatively available in this territory. Underground water for irrigation between 30 and 200 m is obtainable in good quality and quantity for all agricultural production. In some cases higher salt content and iron level appear in underground waters, and beyond these, higher arsenic concentration can be observed probably from geological origin. Main arsenic ion form is arsenate, which was concentrated in living water residues in Pleistocene and Holocene. In these waters arsenic concentration is 20 to 200 µg/L. In Faculty of Horticulture, Kecskemét College, we started our investigations in order to study the arsenic uptake and accumulating properties of different vegetables, grown under the influence of arsenic polluted sprinkling water. Our methods were indoor and outdoor growing, sprinkler and drip irrigation, soil and hydroculture manner as well. This paper summarizes our results on field tomato grown with 0-50-100-200-400-800 µg/L (natural As dose) arsenic polluted sprinkling water, with sprinkler and drip irrigation.

Keywords: arsenic pollution, tomato, field experiment, berry weight, ICP-AES

INTRODUCTION

Arsenic (As) is known for a long time, it is a toxic element in our ground water. It has been proved since the early 1980s that the arsenic content of drinking water in the water base areas of the southeastern counties (Bacs-Kiskun, Békés, Csongrad and Szolnok) exceeds the standards which was 50 µg/l in that time (BARTHA, 2004).

According to current legislation in Hungary the arsenic content of drinking water can be maximum 10 µg/l (201/2001th Government Decree). As for irrigation water the concentration must be below 100 or 200 µg/l (depending on direct or indirect consumption of plants) (MI-10-172/9-1990). If the food is derived from plant or it is consumed as raw food the maximum concentration is 200 µg/ kg (17/1999th (VI.16) EüM). Arsenic is toxic to humans and animals alike, its inorganic forms are seriously toxic, and known as carcinogens. Arsenic causes disease of nervous system, kidney, hematopoietic system, the respiratory system, liver function decreases, reproductive and genetic anomalies also occur. The human body suffers as a whole (COLLECHI ET AL., 1986; KLIMENTNÉ AND MUCSI, 1992).

This problem was recognized in 1981-83. Arsenic is the most severe problem in Hungarian groundwater supplies. Approximately 80% of domestic vegetable-growing area (an average of 50 to 60 thousand ha) that is located in the Southern Great Plain region is affected by arsenic contaminated water. Vegetables that are grown in forced and field

conditions can contact arsenic contaminated irrigation water since they are irrigated with non-purified water.

Arsenic accumulation in plant parts that are intended for consumption is not inevitable. Different plants react for toxic elements in different ways. It is very important to know which vegetable species that are produced in the area may have critical arsenic concentration which is 0.200 mg/kg As relative to the original moisture content of the product.

In this paper the aim of the experiments is

- to investigate the effect of arsenic-treated water on tomato,
- to know what extent this plant takes up arsenic and in what arsenic concentration accumulates in plant parts - leaves, roots, and generative organs - berry-plant parts,
- to know the effect of arsenic contaminated irrigation water on the change of the yield of the tomato berry weight,
- to know if there are visible symptoms on the tomato, due to the arsenic uptake.

MATERIAL AND METHOD

Tomato was analyzed in field. The experiments were set in the Demonstration Garden of the College Faculty of Horticulture of the Kecskemét College in 2011 and 2012. The samples were analyzed in the Soil and Plant Testing Laboratory of the Faculty.

The tomato experiment was performed under field conditions in 2011 and 2012. During the test, the plants were raised in ground containers of 0.3 m^2 surface. The soil of the containers is a fertile sand soil with good humus content. The planting of seedlings was carried out on May 18, 2011 and May 26, 2012. The plants were treated with arsenic treated irrigation water throughout the growing period, arsenic doses employed were as follows: 50, 100, 200, 400 and 800 mg/l. The arsenic was present in the water in forms of arsenic trioxide (As_2O_3) arsenite (H_2AsO_3-) and arsenate (H_2AsO_4-). The water for treatment was prepared in the laboratory.

Two different kinds of irrigation methods were used in the experiments. Sprinkler irrigation released the arsenic irrigation water over the entire surface of the plant, whereas arsenic drip irrigation water was applied only on the soil surface.

Treatments were performed in 4 replicates. In all arsenic dose of both irrigation methods two containers were selected, two tomato plants per container were grown, so the samples of 4-4 plants were constituted in four repetitions.

During the experiment leaf samples were collected several times according to ripeness. Upon the completion of testing root samples were collected.

In the experiments the yield of the plants was determined. In tomato the quantity of berries was weighed.

The dry matter content of the samples was determined in laboratory and after the nitric acid/ hydrogen peroxide digestion of the samples total arsenic content of the samples was analyzed. The measurement was carried out by ICP-AES technique (HÜVELY, 2005).

RESULTS

The arsenic content of tomato roots increased by increasing arsenic doses, in both study years and in each irrigation method. In 2011 a significant difference existed between each

successive dose by the effect of drip irrigation, and the maximum arsenic concentration reached 7.57 mg/kg dry matter. In sprinkler irrigation treatments, except from 2 treatments (100 and 400 mg/l), there was a significant difference between successive doses, too, the maximum arsenic concentration reached 5.47 mg/kg. Regression analysis revealed that the „r" values were high (0.95 and 0.93, P = 1%). In 2012 the tendency was similar, but we measured different arsenic concentration values in the root. The highest measured value was 16.4 mg/kg (in drip irrigation) and 44.5 mg/kg (in sprinkler irrigation), that is there has been a considerable increase in compared to 2011. In the second year the higher concentration was due to the sprinkler irrigation method (*Table 1*).

Table 1. Arsenic concentration in roots and leaves of tomato in 2011 and 2012

As doses	2011				2012			
	Drip irr.		Sprinkler irr.		Drip irr.		Sprinkler irr.	
	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves
Control	0.449	<0.300	0.406	<0.300	<0.300	<0.300	<0.300	<0.300
50 µg/l	1.54	5.62	1.59	5.51	3.03	7.18	2.98	5.78
100 µg/l	2.64	5.77	2.04	7.48	3.59	7.43	5.73	6.74
200 µg/l	3.51	5.25	3.53	10.3	4.92	8.31	11.4	8.49
400 µg/l	6.22	5.83	3.79	11.4	6.88	8.05	18.9	12.0
800 µg/l	7.57	7.05	5.47	16.0	16.4	8.69	44.5	16.5
$SD_{5\%}$	*0.563*	*0.613*	*0.460*	*1.26*	*1.18*	*1.34*	*1.98*	*1.05*
								mg/kg d. m.

In most of the samples the arsenic concentration of tomato leaves increased by the increasing arsenic doses and there were large differences between the results of the irrigation methods.

In drip irrigation, variance analysis justified significant differences only between control treatment and other dose in both study years. The values were between 5.25 and 8.69 mg/kg in dry matter. As the growing season progressed, the leaf arsenic concentration increased steadily in both years according to the difference between control and treatments. The calculated regression coefficient values were between 0.521 and 0.608. It shows that the correlation is not statistically strong. The results of two years are parallel with each other; the highest measured value is 8.69 mg/kg in dry matter.

When sprinkler irrigation method was applied the arsenic content of tomato leaves increased linearly with the increasing dose. In each test periods significant differences ($SD_{5\%}$) existed between the control and the lowest dose, and between 2, 3 and 4 successive dose. R-values of the regression analysis were between 0.843 and 0.951. This proved to be significantly different in four out of the five periods, at P = 1-2% error likelihood. In both years arsenic concentration increased by the effect of sprinkler irrigation too in individual dose, as the season progressed by. It can also be stated that arsenic concentrations of the leaves were generally higher in of sprinkler irrigation then in drip irrigation in both years. The results of the two years were parallel to each other. The maximum arsenic concentration was at sprinkler irrigation, 16.5 mg/kg in dry matter.

The arsenic concentrations of tomato berries were very low. In the processing of the sample, the dry matter content of the berry crop was determined, which allowed the calculation of berry arsenic concentrations in initial moisture content. In the two study years, the arsenic content of the berry changed from 0.022 to 0.080 mg/kg, in the entire dose range. This value is 11-40% of the existing food legislation authorized limit.

In 2011 treatments did not change tomato yield. In 2012, in case of drip irrigation there was no change in the yield too. However, the average berry yield per container reduced significantly under sprinkler irrigation.

CONCLUSIONS

The results of the experiment showed that arsenic concentration grows in the vegetative parts of tomato in significant rate due to arsenic treated irrigation water. The highest concentration was measured in the root samples. Compared to this, the concentration was one or two magnitudes lower in leaves and one magnitude lower in the berries.

Over the time, the test results in field growing conditions show that the arsenic concentration of the matured tomato leaves (the same age) grew increasingly in both drip and sprinkler irrigation by the arsenic treatments. In 2011, in drip irrigation, at 800 mg/l of arsenic concentration treatment, 4.11, 4.52, 7.05 mg/kg arsenic concentrations were measured in the leaves in the consecutive sampling periods. As for sprinkler irrigation, the measured values were 6.60, 13.1, 16.0 mg/kg, respectively. In 2012, in drip irrigation, applying the highest dosage level, the arsenic concentration was 6.28, 8.69 mg/kg in the leaves. In sprinkler irrigation these values were 14.4, and 16.5 mg/kg.

According to the results of this paper, in field experiments sprinkler irrigation - implementing contact arsenic charging – resulted a significantly higher arsenic concentration in tomato leaves than drip irrigation. In 2011, the biggest differences in tomato arsenic concentration, at 800 mg/l of arsenic dose, were 16 and 7.05 mg/kg and in 2012 the differences were 16.5 and 8.69 mg/kg.

On the Southern Great Plain region, either in humic sandy soil or in hydroponics the arsenic concentration in tomato plant parts for consumption does not accumulate over the legal safety limit value (0.200 mg/kg) if the arsenic concentration of irrigation water is about 200 µg/l.

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THE EFFECT OF YEAST CULTURE PRODUCTS (RUMISACC AND INTETOTAL) ON FATTENING PERFORMANCE, SOME BLOOD AND RUMEN FLUID PARAMETERS IN MALE KIDS

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ABSTRACT

The aim of this study was to evaluate the effects of live yeast culture and the combination of live yeast culture with vitamin-mineral supplementation as a feed additive on fattening performance, some blood and rumen fluid parameters in male kids. Totally 18 male Saanen goat kids were divided in to one control and two treatment groups each containing 6 kids. Rations of groups were formulated as isonitrogenic and isocaloric. Live yeast culture (YC) and the combination product (YVM) (RumiSacc® and Intetotal® respectively,by Integro Food Industry and Trade Co., Istanbul, Turkey; Live yeast cell 344 x 10^{10} cfu per gram) was included in the concentrates at 0 (C), 1% (YC) and 1% (YVM) on feed basis, respectively. Feeding schedule was established with only concentrate. Feed was given *ad libitum* and roughage was not given. Dietary yeast culture at the level of 1% increased final live weight (+4.7% regarding control group). All investigated fattening performance with rumen fluid and blood parameters were not statistically affected with the supplements. It is concluded that live yeast culture and its vitamin-mineral combination did not have adverse affect in male kids fed without roughage.

Keywords: Live yeast culture, fattening performance, blood parameters, rumen parameters, kid

INTRODUCTION

Yeast and yeast products are often used in ruminant diets to manipulate rumen fermentation and improve animal performance. The benefits of live yeast culture are well understood however researchs of its products in small ruminants are limited. Studies that have examined effects of yeast cultures have reported variable results. These differences may depend on many factors such as diet composition, forage to concentrate ratio, type of forage feed, yeast dose, feeding strategy and stage of lactation (YALÇIN ET AL., 2011).

Studies reported that yeast supplementation increase growth, feed intake and nutrient utilization in Black Bengal kids (PAL ET AL., 2010), improved feed conversion ratio of Awassi lambs (HADDAD AND GOUSSOUS, 2005), In contrast yeast supplementation did not always improve the animal performance. One of the study on Awassi lambs and Shami goat kids reported, yeast supplementation had no effect on average daily gain and dry matter intake (TITTI ET AL., 2008). One of the study on Saanen dairy goats in early lactation period show that addition of live yeast to diet increased dry matter intake and milk yield (STELLA ET AL., 2007).

Some of studies found that yeast culture supplementation did not affect on some serologycal blood parameters in goats (ÖZSOY ET AL., 2013) and in dairy cows (YALÇIN ET AL., 2011). However GALIP (2006) reported that dietary yeast culture altered serum total protein, urea, calcium concentrations, Ca/creatinine ratio, triglyceride concentrations in rams.

Addition of yeast culture in diets of ruminants had conflictual results on rumen fatty acid (VFA) concentration. One of the report on dairy cows with two different saccaromyces strain indicated that strain has an importance and that can be modify ruminal ammonia, propionate and butirate concentration, however have no effect on productive performance (PINOS-RODRIGUEZ ET AL., 2008). Also another investigation (DOLEZAL ET AL., 2005) in which the effect of addition of a yeast culture (*Saccharomyces cerevisiae*) on rumen fermentation in dairy cows indicated a positive effect on production of VFA. DESNOYERS ET AL. (2009), reported that the positive effect of yeast supplementation on rumen pH increased with the percentage of concentrate in the diet and with the dry matter intake level. They also indicated that the positive effect of yeast supplementation on rumen VFA concentration increased with dry matter intake and crude protein levels. Related with active dried yeasts in young ruminants, CHAUCHEYRAS-DURAND ET AL. (2008) also mentioned that yeast have a stabilization function on rumen pH.

The goat population was about 5 million in Turkey and one of the prefered dairy goat species is Saanen. Male kids have less economic value for dairy farms in birth season compared with females. Also there is growing concern on combination feed additives because of economic reasons. Live yeast vitamin-mineral combination is one of it. Effects of supplementing live yeast culture (Rumisacc® İntegro Gıda AŞ, Turkey) and the combination of live yeast culture with vitamin-mineral (Intetotal® İntegro Gıda AŞ, Turkey) to concentrate rations fed to fattening male saanen kids have not been studied. Therefore, the objective of this study was to evaluate the effects of these products supplementation to fattening diets of male Saanen kids on feed intake, growth performance, and some blood parameter and ruminal volatile fatty acids.

MATERIAL AND METHOD

Investigation was started with the permission of animal experiments local ethical committee of Mehmet Akif Ersoy University. A total of 18 male Saanen kids aged 2 months were used at the study. All the animals were treated for internal and external parasites using Ivomec (Novakim; active ingredient: 10 mg/ml Ivermectin; dose: 1ml/50 kg live weight) 2 weeks before the experiment started. This study was conducted at the commercial feedlot for 11 weeks from May 2013 to July 2013. Kids were housed individual cages (2m x 3m) under the shed with concrete floor with sawdust and dry manure bedding for the entire period of the experiment. Concentrates were prepared in a feedmill as a mash feed. The rations were formulated to be isocaloric and isonitrogenous. The ingredients and the chemical composition of the concentrates are presented in *Table 1.* Live yeast culture and its vitamin-mineral combination product (RumiSacc® and Intetotal® respectively, Integro food Industry and Trade Co., Istanbul, Turkey) were both included in the concentrates at 1,0%. Cu concentration of YVM (Intetotal®) decreased by producer because of mineral concentration of experimental ration. During the study concantrates and fresh water were given *ad libitum* and the ration was not contain roughage. Feed refusals were collected once a week and weighed to accurately determine feed intake.

Nutrient composition of concentrates, live yeast culture and its vitamin-mineral combination product were determined according to the AOAC (2000).The metabolizable energy levels of concentrate feeds were determined by using the following formula of TSI (1991).

ME (kcal/kg OM) = 3260 + (0.455xCP) – (4.037xCF) + (3.517xEE) where CP (crude protein), CF (crude fibre) and EE (ether extract) were expressed as g/kg OM (organic matter) and converted dry matter (DM) basis.

Table 1. The ingredients and chemical composition of the concentrate feeds

Ingredients, % as feed basis	Dietary treatments		
	C	YC	YVM
Corn	35	35	35
Barley	21	21	21
Wheat Bran,	11	11	11
Full fat soy	11	11	11
Sunflower meal, 36% Crude Protein	10	10	10
Soybean meal, 48% Crude protein	7	6	6
DCP	1.7	1.7	1.7
Canola oil	1	1	1
DL-methionine	0.1	0.1	0.1
L-Lizin hidrochloride	0.1	0.1	0.1
Live yeast culture[1]	-	1	-
Live yeast – vitamin,mineral combination[2]	-	-	1
Lime stone	1.5	1.5	1.5
Salt	0.4	0.4	0.4
Vitamin mineral premix[3]	0.2	0.2	0.2
Analysed composition, % as feed basis			
Dry matter, %	88.38	88.40	88.09
Crude protein, %	16.34	16.68	16.38
Ether extract, %	5.31	5.94	6.06
ME, kcal/kg ME	2648.21	2674.35	2668.53

C: Control group; YC:group fed with diet containing live yeast culture;YVM: group fed with died containing the combination of live yeast culture with vitamin and mineral, 1: RumiSacc, Integro Food Industry and Trade Co., İstanbul, Turkey, 2: Intetotal, Integro Food Industry and Trade Co., İstanbul, Turkey, 3: Each kilogram of vitamin-mineral mix contains 12 000 000 IU A vit, 20 000 mg E vit, 50 000 mg Mn, 50 000 mg Fe, 50 000 mg Zn, 10 000 mg Cu, 800 mg I, 150 mg Co, 150 mg Se

Animals were individually weighed at the beginning of the experiment and every two weeks. The average daily weight gain over the duration of experiment was determined individually. Daily dry matter intakes of the kids were determined and feed conversion ratio was calculated as kg feed per kg live weight gain of kids individually.

Rumen fluid samples were collected in two bottles from all kids in each group during the slaughtering process. One bottle of rumen fluid sample was used for the measurement of pH and the other one was for VFA. The pH was measured immediately by a pH meter (Hanna pH meter, model no: Hi917hN). Rumen fluid samples were filtered from cheese cloth before VFA analysis. After centrifugalize (10.000 rpm, 10 min at +4°C) concentrations of VFA in the supernatant were determined by HPLC system of Agillent 1260 series (Agillent Technologies, Waldronn, Germany) equipped with a Agilent-detector (1260 MVDVL) operated at 210 nm. Separation of acids was conducted using an organic acid analysis column (300 x 7.7 mm; Hi-plexH-organic acid column), with 0.005 M H_2SO_4 as eluent, at flow rate of 0.6 ml/min, and with the column temperature of 55°C. Concentrations of ammonia-N were determined by distillation (Gerhard, vapodest 2000) and titration, by using 5 ml of the rumen fluid which filtered by cheese cloth (ANONYMOUS, 2014).

Blood samples were taken in two tubes from jugular vein containing EDTA for hematological analysis and without EDTA for biochemical analyzes with the aid of the cannula at the last day of the experiment. Tubes for biochemical analysis were centrifuged at 3000 rpm at room temperature for 5 minutes and then serum was carefully harvested for determination of total cholesterol, triglyceride, glikoz and blood urea nitrogen (BUN) were analyzed by VET TEST 8008 Autoanalyzer (IDEXX Laboratories, inc Westbrook ME

04092 USA). Other blood samples were freshly used for hematological analyzes (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDWc) were analized by Abacus Junior Vet Hematology Analyzer (Diatron MI PLC. Hungary).

Statistical analysis have done using computer programme. One way ANOVA was performed to detect the differences among groups. The significance of mean differences between groups were tested by Tukey (DAWSON AND TRAPP 2001). Values were given as mean ± standard error. Level of significance was taken as $P<0.05$.

RESULTS

Analysis of Rumisacc® and Intetotal® is showed that these additives are rich in protein, they contained 44.31 and 40.51% CP respectively. Both of two dietary live yeast additive did not significantly affect for the final live weights of kids (*Table 2*). Average body weight gain, feed intake , feed convertson ratio (*Table 3*), hot and cold carcass yield (*Table 4*), ruminal ammonia-N and VFA (total and individual) concentration with Ammonia-N (*Table 5*), VFA (total and individual) concentration (*Table 6*), Initial and final hematological and blood chemistry results (*Table 7* and *Table 8*) were not significantly affected ($p>0.05$) by treatment of groups.

Table 2. Effects of dietary treatments on body weight of kids, kg

	Dietary treatments			
	C	YC	YVM	p
Initial body weight,kg	16.45 ± 0.82	16.80 ± 0.35	16.82 ± 1.22	0.939
Day 14	19.39 ± 0.86	20.03 ± 0.45	19.29 ± 1.26	0.808
Day 28	22.16 ± 1.21	22.12 ± 0.35	22.41 ± 1.49	0.866
Day 42	24.20 ±1.44	25.57 ± 0.54	24.44 ± 1.43	0.683
Day 56	27.67 ± 1.64	29.02 ± 0.47	27.43 ± 1.48	0.654
Day 70	30.15 ± 1.71	31.68 ± 0.52	29.91 ± 1.70	0.629
Day 77	30.68 ± 1.83	32.12 ± 0.52	30.98 ± 2.06	0.785

n=6, $p<0.05$

Table 3. Effects of dietary treatments on performance parameters

	Dietary treatments			
Average results	C	YC	YVM	p
Weight gain, g/d.	175.67 ± 16.59	187.59 ± 12.66	181.30 ± 22.65	0.881
Feed intake, g/d.	952.06 ± 50.57	1055.72 ± 22.33	1013.05 ± 68.96	0.325
Feed conversion ratio,(feed intake/ weight gain)	5.53 ± 0.27	5,78 ± 0,48	5,79 ± 0.53	0.889

n=6, $p<0.05$

Table 4. Effects of dietary treatments on hot and cold carcass yield

	Dietary treatments			
Item	C	YC	YVM	p
Hot carcass weight, kg	14.30 ± 0.83	14.70 ± 0.28	13.80 ± 1.00	0.706
Cold carcass weight, kg	13.86 ± 0.81	14.33 ± 0.29	13.48 ± 0.96	0.717
Hot carcass yield, %	46.62 ± 0.62	45.75 ± 0.44	44.48 ± 0.86	0.104
Cold carcass yield, %	45.21 ± 0.77	44.61 ± 0.50	43.46 ± 0.75	0.236

n=6, $p<0.05$

Table 5. Effects of dietary treatments on rumen pH and rumen -N

Rumen	Dietary treatments			
	C	YC	YVM	p
pH	5.50 ± 0.08	5.64 ± 0.07	5.53 ± 0.15	0.602
NH3-N ,mg/l	1079.33 ± 190.90	991.00 ± 53.31	992.00 ± 50.10	0.101

n=6, p<0.05

Table 6. Effects of dietary treatments on rumen volatile fatty acids (mg/l)

Item	Dietary treatments			
	C	YC	YVM	p
Lactic acid	404.89 ± 192.08	7.97 ± 5.87	70.87 ± 51.07	0.720
Acetic acid	6462.00 ± 562.66	6061.50 ± 549.76	5151.40± 515.71	0.275
Propionic acid	4315.84 ± 622.55	3365.05 ± 638.59	2260.85± 431.83	0.087
Iso-butyric acid	460.97 ± 133.15	288.39 ± 69.77	190.84 ± 83.11	0.203
n- butyric acid	1449.45 ± 211.39	1416.18 ± 178.83	1693.09± 237.20	0.620

n=6, p<0.05

Table 7. Initial hematological and blood chemistry results of kids

Item	Dietary treatment			
	C	YC	YVM	p
WBC, 10 /L	11.82 ± 1,14	12.54 ± 1,85	12.10 ± 1,17	0.937
RBC, 1012/L	17.31 ± 0,39	17.60 ± 0,42	17.85 ± 0,49	0.697
HGB, g/dl	9.21 ± 0,23	9.45 ± 0,28	9.16 ± 0,43	0.792
HCT, %	24.35±0.62	24.67 ± 0.68	23.92 ± 1.11	0.811
MCV, fl	14.00 ± 0.44	14.00 ± 0.44	13.40 ± 0.50	0.607
MCH, Pg	5.31 ± 0.13	5.36 ± 0.14	5.14 ± 0.20	0.487
MCHC, g/dl	37.86 ± 0.70	38.21 ± 0.34	38.32 ± 0.50	0.829
RDWc, %	46.60 ± 1.07	46.11 ± 1.20	47.10 ± 0.69	0.813
Total cholesterol, mmol/L	2.89 ± 0.40	2.42 ± 0.48	3.46 ± 0.42	0.298
Glucose, mmol/L	5.77 ± 0.24	5.73 ± 0.52	6.53 ± 0.50	0.395
BUN, mmol/L	3.28 ± 0.38	5.90 ± 0.98	4.48 ± 1.23	0.143
Triglycerides, mmol/L	0.22 ± 0.10	0.26 ± 0.14	0.33 ± 0.24	0.221

n=6, p<0.05

Table 8. Final hematological and blood chemistry results of kids

Item	Dietary treatment			
	C	YC	YVM	p
WBC, 10 /L	12.09 ± 1.46	13.39 ± 1.16	12.75 ± 0.71	0.739
RBC, 1012/L	17.35 ± 0.53	17.66 ± 0.49	17.77 ± 0.41	0.825
HGB, g/dl	9.21 ± 0.25	9.66 ± 0.22	9.54 ± 0.63	0.685
HCT, %	23.21 ± 0.55	24.11 ± 0.76	22.95 ± 1.41	0.655
MCV, fl	13.16 ± 0.30	13.83 ± 0.47	13.00± 0.70	0.474
MCH, Pg	5.31 ± 0.60	5.46 ± 0.13	5.36 ± 0.27	0.797
MCHC, g/dl	39.71 ± 0.43	40.11 ± 0.43	41.44 ± 0.76	0.107
RDWc, %	45.50 ± 0.14	44.61 ± 0.56	46.72 ± 1.04	0.108
Total cholesterol, mmol/L	2.17 ± 0.20	2.31 ± 0.34	1.49 ± 0.31	0.160
Glucose, mmol/L	3.64 ± 0.14	3.67 ± 0.10	3.65 ± 0.14	0.989
BUN, mmol/L	7.38 ± 0.53	7.43 ± 0.66	7.54 ± 0.56	0.983
Triglycerides, mmol/L	0.25 ± 0.01	0.29 ± 0.02	0.26 ± 0.04	0.475

n=6, p<0.05

DISCUSSION AND CONCLUSIONS

Supplementation of YC increased final weight up to 1.44 kg more without significant difference compared with control group. This case may be attributed to low numbers of animals and individual differences in body weight of the animals in the groups. It is interesting that the average daily weight gain was approximately two times higher in YVM group than in other treated groups at the final week of the experiment. Related with the average body weight gain results of this study is similar with TITTI ET AL., (2008). The investigators reported that yeast culture supplementation did not affect for the live weight, live weight gain and dry matter intake in Ivesi lambs and Shami goat kids. On the other hand significant increases in live weight gain associated with yeast supplementation have been reported in goats (KAMAL ET AL., 2013; ÖZSOY ET AL., 2013) and lambs (HADDAD AND GOUSSOUS, 2005). In the present study the kids fed diet containing either two yeast culture product consumed 11.7 and 6.38% more feed dry matter respectively than control group. Beside this result, KAMAL ET AL. (2013) reported that live yeast supplementation improved significantly the dry matter intake (DMI) per kg gain. Investigators also mentioned that more DMI and relatively more average daily gain in leave yeast fed groups subsequently lead to the improvement of the feed conversion ratio at the same study. There are several studies which have mentioned improvement in feed conversion ratio due to yeast feeding of lambs (HADDAD AND GOUSSOUS, 2005) and in goats (JINTURKAR ET AL., 2009). However TITTI ET AL. (2008), reported that yeast culture supplementation increased digestibility with no effect on growth, feed intake or feed convertion ratio of fattening Awassi lambs and Shami kids.

Very little published literature is available concerning effects of yeast culture supplementation on carcass, especially with small ruminants. TITTI ET AL., (2008), reported that yeast culture supplementation significantly decreased cold dressing proportion and hot carcass weight of Awassi lambs however did not affect on Shami goat kids as our results.

Our ruminal pH results are similar with a series of study which have shown that ruminal pH was not affected by the supplementation of *Saccharomyces cerevisiae* (GARCIA ET AL., 2000; GALIP, 2006; KAMAL ET AL., 2013). However significant increase of ruminal pH associated with yeast supplementation, have been reported in goats (ABD EL-GHANI, 2004; ÖZSOY ET AL., 2013). In the present study kids were adapted to concentrate in early age. This situation may have influence for stability of ruminal pH. Also there are investigations which have similar result (AYDIN ET AL., 2003; MOYA ET AL., 2009) are available related with *Saccharomyces cerevisiae* and ruminal fluid of ammonia-N concentration with our results. However ÖZSOY ET AL. (2013), reported that dietary inclusion of 3.0 and 4.5% live yeast culture significantly increased ammonia-N concentration on goats. Similarly, GALIP (2006a), indicated that ruminal ammonia-N concentrations increased significantly by dietary yeast culture supplementation whatever the ratio forage/concentrate of the diet. Related with VFA concentrations of ruminal fluid there is a series of study (GARCIA ET AL., 2000; AYDIN ET AL., 2003; ÖZSOY ET AL., 2013) which have similar results with ours. However KAMAL ET AL., (2013) indicated that total volatile fatty acid concentration was significantly higher in live yeast culture fed kids at 2 and 4 months.

Blood chemistry results and some hematologycal parameter results of present study had parallel with ÖZSOY ET AL. (2013). They reported that plasma cholesterol and trigliceride concentrations were not altered by yeast culture supplementation on goats and YALÇIN ET AL. (2011) on dairy cows. On the other hand, dietary yeast supplementation did not change serum trigliceride and cholesterol levels in rams (GALIP, 2006).

Addition of live yeast culture and its vitamin mineral combination to male kids fed with concentrate (without forage) did not affect for the investigated parameters significantly.

Dietary yeast culture at the level of 1% increased the final live weight (4.7%) compared with control group.

More research needs with different doses and more replicates to be conducted to determine the affects of live yeast culture products.

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ROLE OF GENEBANK IN MAIZE (*ZEA MAYS* L.) AND WHEAT (*TRITICUM AESTIVUM* L.) BREEDING

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ABSTRACT

Maize breeding In the last three decades, a large number of maize hybrids have been developed from genotypes with a restricted genetic base. In order to decrease the genetic vulnerability, it is very important to widen the genetic base for maize breeding by the application of various methods. One particular way to increase genetic variability is treatment with various mutagens. After more than twenty-five years of research, it has been proved that such lines can be produced by mutation. In 1995, a maize gene bank was established with 1,500 lines in our department. There is large genetic variation in the maize gene bank, the exploitation of which is only possible using suitable methods of selection and evaluation. As a result of mutation, the new maize inbred lines P26, P61 and P62 were released after DUS tests. Radiation generated conspicuous changes in the plant characteristics. The most pronounced aberrations were observed for the expression of anthocyanin coloration and flowering time in various plant organs.

Wheat breeding The study was designed to examine the effects of four sucrose concentrations (45, 60, 75 and 90 gL^{-1}) and four maltose concentrations (65, 100, 135, 170 gL^{-1}) on callus induction, plant regeneration and green plant proportions. Anthers of Pavon 76 were cultured on these induction media and embryoids induced were transferred to the standard regeneration medium to compare for differences in green plant percentage and the influence of carbon sources on it. The green plant ratio showed a linear correlation with the concentration, being the highest (22.3 green plants/100 cultured calli) at 90 gL^{-1} fructose and that (31,7%) at 170 gL^{-1} maltose.

Keywords: genetic diversity, maize (*Zea mays* L.), mutation breeding, winter wheat, plant regeneration

INTRODUCTION

Maize breeding Since the 1920s, the hybrid maize production in the world has been based on the development and crossing of inbred lines. Currently in maize, many new hybrids have been developed from crosses of a limited number of parent lines. This represents a great risk for the loss of genetic diversity in elite germplasm (HALLAUER ET AL., 1988; cit. MESSMER ET AL., 1993). For these reasons and others, maize breeders have a keen interest in the characterization of genetic diversity among parental lines. The genetic diversity among breeding materials could help to prevent the great risk of increasing uniformity in the germplasm and could also ensure long-term selection gains. Due to specific breeding aims and gene erosion, the genetic basis of maize breeding has decreased significantly in Hungary in recent decades, with well-known unfavourable effects (TÓTH AND PEPÓ, 2003). To select maize varieties with more favourable genetic structures, it is necessary to enlarge the basic genetic materials and enrich the available gene sources (PÁSZTOR, 1992). The success of plant breeding depends mainly on the genetic diversity of the basic material. Crossing and mutation are different methods which are applied to generate genetic diversity (HAJÓS NOVÁK ET AL., 1996). According to RADY AND NAGY (1996), in the interests of greater yield stability, the aim is for each maturity group in each growing area to be represented by hybrids of different genetic origins. When creating hybrids, lines with diverse genetic origin should be used in order to achieve a greater heterosis effect (RADY AND NAGY, 1996).

In recent years, maize hybrids have been highly productive, but their reduced genetic variability leave them with a reduced capacity to deal with new diseases and pests (e.g. as the European corn borer in the 1940s and 1950s or southern corn leaf blight in 1970 in the U.S. Corn Belt (DUVICK AND CASSMAN, 1999), and other changes in environmental conditions. Sustainable agriculture is not new in maize breeding, most of the modern hybrids can utilize fewer external inputs (e.g. pesticides, fertilizers) by high productivity, and caused minimal environmental impacts. However with the integration of *in vivo* and *in vitro* techniques in maize breeding programs, we can obtain desirable agronomic attributes, accelerate the breeding process and enhance the genes responsible for them (PEPO, 2004).
The aim of this study was to reveal genetic variability in inbred maize lines (P26, P61, P62) produced by physical mutagens (fast neutron irradation) on the basis of morphological characteristics (expression of anthocyanin coloration, flowering time).

Wheat breeding In wheat, the low level of callus induction from microspores and subsequent plant regeneration, and high percentages of albino plants *in vitro* limited the application of haploids in plant breeding and genetic research for cereal crops (QUYANG, 1986). The yield of green haploid plants in anther culture depends on three independent components: embryoid production from cultured anthers, plant regeneration from the embryoids and the percentage of green plants (SZAKACS ET AL., 1988). Researchers have therefore shifted their efforts to investigating the influence of medium components on the proportion of green plants produced (BJORNSTADT ET AL., 1989; ZHOU, 1990). Sucrose as osmotic agent not only acts as a common source of carbon in the cell culture media of cereal (AL-KHAYRI AND AL-BAHRANY, 2002) and energy but also as an osmoticum during organogenesis (HUANG AND LIU, 2002) and accumulated in many plant tissues in response to environmental stress, including water deficit (RAMOS ET AL., 1999) for playing a role in osmoregulation and cryoprotection. It has also been reported that sucrose in lower concentration (2% and 4%), is necessary for optimal growth and multiplication (HAZARIKA, 2003).

MATERIAL AND METHOD

Maize breeding

The story and establishment of a maize gene bank by mutation in Debrecen

In Debrecen, mutation breeding was initiated in 1960 by Károly Pásztor. Within the programme started in 1979-80, F1 maize hybrid seeds were treated by radiation of Co60 isotope. Later, in 1985 and in 1991, the trial was expanded and the seeds were treated by radiation of fast neutrons at the Atomic Research Institute of the Hungarian Academy of Sciences in Debrecen. The selected mutant lines - developed in this way - have been self - pollinated for several years. In 1995, a maize gene bank was established with 1,500 lines, which are registered by the IBPGR (FRISON AND SERWINSKI, 1995). The experiment was set in the demonstration garden of the Department of Genetics and Plant Breeding in the Agricultural Centre, at the University of Debrecen.

Importance of induced mutagenesis today

Induced mutation has become an effective tool for plant improvement and has also increased bio-diversity. According to the FAO/IAEA data in 2001, the quantity of new plant varieties selected by mutation breeding has been increasing from year to year. As of June 2000, 2,252 new mutant varieties have been released in 50 countries all over the world. These new plant varieties originated from 154 plant species, and 1,275 of the 2,252

new mutant plants are agricultural plant varieties, mostly cereals (rice, barley, wheat and maize). In reality, it would be expected that the number of released varieties is much higher than listed, as many mutated genes have been used in cross breeding programmes without indicating the nature of the desired genes (MALUSZYNSKI ET AL., 2001).

Wheat breeding

A spring wheat cultivar, 'Pavon 76' was used as donor material. Liquid potato 4 (P4) medium (OUYANG, 1986) was used as a standard induction medium. Spikes were selected at random from a bulk from which 100 anthers were excised for each petri dish, with four replicates per treatment. The callus induction frequency was the number of calli obtained from 100 anthers plated 40 d following induction. Embryoids induced from the anthers were transferred to a '190-2' medium for plant regeneration when they reached 1 mm in diameter. The standard regeneration medium contained 30 gL^{-1} sucrose and was solidified using 6 gL^{-1} agar. No plant growth regulator (PGR) was included in the regeneration medium. The time of transfer was based on the size of the calli that developed between 30 to 40 d after anther plating. After 30 d of regeneration culture the number of calli that developed into albino or green plants was recorded. Plant regeneration frequency was the number of calli producing plantlets per 100 calli transferred. The green plant regeneration frequency was the number of calli producing green plantlets divided by the number of total calli producing either green or albino plants.

RESULTS

Maize breeding

Heterosis breeding in maize has caused gene erosion by using uniform inbred lines (PEPÓ AND TÓTH, 2004). In order to avoid this, an *in vivo* maize gene bank has been established which contains inbred lines with greater genetic potential and better ecological adaptation. After more than twenty-five years of research, it has been proved that such lines can be produced by mutation. The most important data of the gene bank are shown in *Table 1.*

Table 1. Number of inbred lines in the maize gene bank produced by mutation

Lines	Years									
	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
Characterization*	155	155	155	155	155	155	122	106	92	90
Maintenance**	517	450	450	450	217	217	250	310	120	102

*: According to UPOV
**:By self-pollination

Table 2. Pedigree of registered inbred lines

Lines	Hybrids	Type of irradation	Dose [Gy]
P26	**F_1(Pi 3747 SC) M_2**	fn	7.5
P61	**F_1(Pi 3901 SC) M_2**	fn	12.5
P62	**F_1(Pi 3901 SC) M_3**	fn	7.5

F_1 : First generation after crosses
M_n : n^{th} mutational generation
fn : Fast neutrons (produced in a cyclotron)

There is large genetic variation in the germplasm utilised, the exploitation of which is only possible using suitable methods of selection and evaluation. In 1985, we applied a physical mutant agent (fast neutron) for irradiation of F_1 hybrid seeds by 7.5-12.5 Gy dose. After selection, mutant lines were used for self-pollination over many years. As a result of this

selection process, P26, P61 and P62 lines have been governmentally released by the OMMI (2001). These lines serve as a basic material in our future breeding programs. Identification and the origin of these genotypes are given in *Table 2.*

As a result of mutagenic treatment, morphologically very different mutant populations were obtained. Radiation generated conspicuous changes in the plant characteristics described by UPOV in comparison with the basic material (*Table 3*).

Table 3. Comparison of characteristics (UPOV TG/2/6) of basic hybrids and the mutant registered lines (P26, P61, P62) derived from them

Basic hybrids, irradiated lines		Characteristics Code denoting degree of expression of the characteristics				
		Intensity of anthocyanin coloration of silks	Anthocyanin coloration of anthers	Anthocyanin coloration of glumes excluding base	Anthocyanin coloration at base of glume	Flowering time
Initial stock	F_1(Pi 3747 SC) M_2	5	7	7	1	72
Irradiated stock (fn 7.5 Gy)	P26	1	1	1	1	69
Initial stock	F_1(Pi 3901 SC) M_2	5	9	9	1	71
Irradiated stock (fn 12.5 Gy)	P61	1	5	5	1	68
Initial stock	F_1(Pi 3901 SC) M_3	7	7	7	9	70
Irradiated stock (fn 7.5 Gy)	P62	1	5	5	1	68

The most pronounced aberrations were observed for the expression of anthocyanin coloration in various plant organs. The variability manifested in the changes of pollination interval. Flowering time is considered to be quantitatively inherited, and different studies have identified loci that affect this trait in maize (BEAVES ET AL., 1991; cit. OLIVEIRA ET AL., 2004). Mutation treatments (fast neutron) induced earliness in flowering time of different inbred lines. Using these lines as crossing parents, they could cause earliness in hybrids. With earlier flowering time, we can avoid frequent drought periods, which reduce fertilization in maize. Earlier maturity could reduce grain moisture in harvest time, drying energy and fungi diseases (e.g. *Fusarium* ear rot). These characteristics are suitable for sustainable agriculture. We concluded that the cyclotron can be successfully applied in widening genetic variability. We produced a number of inbred lines with wide genetic variability using mutation breeding. We can use this information to develop maize hybrids, which can be useful in our breeding program.

Wheat breeding

The callus induction response to sucrose for v. 'Pavon 76' was higher at all sucrose levels than that of maltose (*Table 1*).

Table 1. *In vitro* green plant proportion in cv 'Pavon 76'

Induction medium g L^{-1}	% of green plants (green plant/No of embryoids)*	Plantlet No/100 calli transferred*	Green plant No/100 calli transferred*
Sucrose 45	22.3 a	50.4 a	11.2 a
Sucrose 60	29.5 b	50.7 a	15.0 b
Sucrose 75	37.3 c	50.8 a	20.6 c
Sucrose 90	42.9 c	51.4 a	22.3 c
Maltose 65	25.0 a	40.9 a	10.2 a
Maltose 100	39.1 b	45.8 b	20.3 b
Maltose 135	64.5 c	47.7 b	30.8 c
Maltose 170	66.0 c	48.0 b	31.7 c

*Values followed by the same letter are not significantly different according to the protected LSD test at P = 0,05.

Pavon 76 showed basically linear responses to sucrose and maltose concentrations. Some trends emerged from the response for callus induction across increasing sucrose and maltose concentrations: (i) the callus induction increased dramatically for both induction media when either sucrose or maltose were increased from 45 to 90 gL^{-1} and from 65 to 170 gL^{-1}, respectively, (ii) callus induction from Pavon 76 can be increased at sucrose levels > 90 gL^{-1} and at maltose levels > 135 gL^{-1} significantly in the induction medium. 66.0% embryoids from the 170 gL^{-1} and 64.5% embryoids from the 135 gL^{-1} maltose medium produced green plants, whereas only 42.9% embryoids from the 90 gL^{-1} sucrose medium produced green plants (*Table 1*). Sugar has two functions in culture media, as a carbon source and as an osmotic regulator. Since the sugar content of media was found to change little during the culture period, the major function of sugar may be as regulator of medium osmolarity. Thus, the difference in green plant percentages between maltose concentrations demonstrates the effect of medium osmolality on albinism. Sucrose in induction media is rapidly hydrolyzed to fructose and glucose, increasing the medium osmolarity, whereas no detectable osmotic change occurs in maltose containing medium.
A distinct response pattern types were found for plant regeneration across increasing concentration in the induction medium; the plant regeneration for 100 calli transferred was 50.4-51.4% in medium containing various amount of sucrose and 40.9-48.0% for that with maltose (*Table 1*). However, the green plant proportion showed a linear response with concentration and was the highest one for sucrose (22.3 green plant number/100 calli transferred) at 90 gL^{-1}, and that for maltose (31.7%) at 170 gL^{-1}, which were not significantly different from 75 gL^{-1} sucrose and 135 gL^{-1} maltose concentration, respectively.

CONCLUSIONS

Maize breeding

In 1995, a maize gene bank was established with 1,500 lines in our department. There is large genetic variation in the maize gene bank, the exploitation of which is only possible using suitable methods of selection and evaluation. As a result of mutation, the new maize inbred lines P26, P61 and P62 were released after DUS tests. Radiation generated conspicuous changes in the plant characteristics. The most pronounced aberrations were observed for the expression of anthocyanin coloration and flowering time in various plant organs.

Wheat breeding

Results from this study have profound implications on the choice of carbon sources and concentrations. Many of the reported effects of medium modifications and pretreatments may be related to osmotic potential. In addition, if the hypothesis about the importance of medium osmotic potential is correct, more attention should also be paid to establishing the optimal osmotic potential for regeneration media. Currently, most researchers use 90 gL^{-1} sucrose in incubation media, but only 30 gL^{-1} sucrose in regeneration media. Because of the hydrolysis of sucrose in induction media, difference in osmotic potentials between the induction and regeneration media during the transition phase of culture is much greater than expected. This difference also may have a significant impact on green plant percentage.

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THE INFLUENCE OF FEED PROTEIN LEVEL ON SOME PRODUCTIVE INDICES IN BARRED PLYMOUTH ROCK REARED IN FREE RANGE SYSTEM

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ABSTRACT
The purpose of this paper was to assess the possibility of grow mixed breed broilers in free range system fed according to the slow feeding rate, with nutritional requirements mainly consisting of feed concentrate mixtures starting from the premises that currently there are no nutritional standards especially created for maintenance alternative systems. The experiment for the quantification of impact of nutritional features and of the CM administration intake on bio productive and economic performances of Barred Plymouth Rock avian youth has been reared during 10 weeks on two experimental variants. The elaborated experimental design was intended to assess the effect of a good nutritional start in both variants, but with a different time. Considering the same start, both in V_1 and V_2, by administering an CM with 2960 kcal ME and 22.04% CP, for 14 days in V_1 and 21 days in V_2.V_1 received a tri-phase feeding by using an intermediary „growing" phase during 36 days when CM was administered with 2990 kcal ME/kg and 20.03% CP followed by a finishing phase after 50 days and continued until the end of experiment, when CM was administered with 3000 kcal ME/kg and 17.30% CP. V_2 was intended to establish the effect of a bi-phase feeding, therefore phase II became the „growing-finishing" phase, respectively the administration of a feed concentrate mixture with an energy level of 3000 kcal ME and 17.30% CP. Broilers in V_1, tri-phase fed, had a feed intake comparison with that of broilers in V_2 (bi-phase fed), an average body weight of 1428.60 g with a total increase of 8.3% higher and a better feed conversion (2.66 kg/kg) considering the increased costs per kg of live mass with 1.87% comparing with V_2. Regarding the CP intake, depending on ME intake, the registered values are close in both variants. Based on a mathematic model like: $y = a/(1+bx+cx^2)$, one can assess the fodder feeding costs. The correlation rate between the fodder feeding costs and the CP intake is strongly positive for both variants.

Key words: protein, costs of feeding, free range system, Ross 308

INTRODUCTION

In most developed countries chicken meat production is more and more based on autochthon ecotypes and alternative range systems. In western countries, the militants for animal welfare lead an ample campaign against intensive production (SAVOY, 2003; KINGORI ET AL., 2007).

VERBEKE AND VIANE (2000), BLOKHUIS ET AL. (2000) state that society segments have shown great interest in production systems, animal welfare and quality of life. That led to a guarantee to consumers' choices of new products (FRASER, 2001).

Improvement in the performance of indigenous birds on free-range requires some knowledge of feed available to them under the prevailing system of production (WALKER AND GORDON, 2003). This will allow an evaluation of their nutritional status and a possible formulation of a supplementary package (KINGORI ET AL. 2007).

Regardless of the range system, feeding is a determinant factor in increasing animal production, being greatly responsible for poultry breeding and development, for maintaining them healthy and for achieving the goal for which they are raised, respectively for providing meat or eggs production.

After a comprehensive study, BLAIR (2008) stated that currently there are no nutritional standards especially created for poultry raised in alternative systems. Yet, those standards can derivate from the already existent regulations provided for genotypes with a slow rate of breeding. Many producers use traditional breeds and genotypes of poultry which were not subject to the selection pressure (SIMIZ, 2012).

BLAIR (2008) states that the ARC (1975) system has applicability in the production obtained in alternative breeding systems, due to their basic genotype, but the data are although incomplete. They suggest as basis for setting certain nutritional standards applicable for poultry in order to produce meat worldwide, NRC (1994) regulations.

The application of these nutritional standards aims to provide a balanced diet containing nutritional principles in specific ratios and they do not contain excess nutrients (DRINCEANU ET AL., 2010; SIMIZ ET AL., 2013).

The purpose of this experiment was to assess the possibility of raising mixed breed chickens for meat production in free range system fed according to the slow growing rate, with nutrient requirements mainly consisting feed concentrate mixtures. The Barred Plymouth Rock variety as a biological material was selected starting from the premise that it is very prevalent in our country and in other countries as well, and it is suitable both for industrial rearing as broiler mother (Plymouth Rock, white variety) and for household rearing due to its resistance.

MATERIAL AND METHOD

The experiment for the quantification of the effect of nutritional features and the concentrate mixtures (CM) intake time on bio productive performances of Plymouth Rock avian youth was performed during 10 weeks in a family farm.

The experiment was performed on a group of 40 broilers under feeding conditions specific to the free range system. Broilers were divided into two experimental variants, as follows:

– ***V_1*** – consisting of 20 broilers tri-phase fed with concentrate mixtures noted with $CM_{starter}$, $CM_{rearing}$ and $CM_{finishing}$;

– ***V_2*** – consisting of 20 broilers bi-phase fed with $CM_{starter}$ and $CM_{rearing\text{-}finishing}$;

Regarding the concentrate mixtures intake, there are three phases in experimental variant 1 and two phases in experimental variant 2.

Phase I: ***Starter*** from day 1 to day 14 in V_1, and from day 1 to day 21 in V_2 ($CM_{starter}$ with 2960 kcal ME and 22.04% CP);

Phase II: ***Rearing*** from day 15 to day 49 in V_1 ($CM_{rearing}$ with 2990 kcal ME and 20.03% CP), in V_2 this intermediary phase is missing;

Phase III: ***Finishing*** from day 50 to day 70 in V_1, ($CM_{finishing}$ with 3000 kcal ME and 17.30% CP) was administered. In V_2, the **rearing-finishing** phase continued 49 days, from 22 days to 70 days while broilers fed $CM_{finishing}$ feed.

The experimental design intended to assess the effect of a good nutritional start in both experimental variants, but with a different time.

Regarding the structures of concentrate mixtures, there were the following assessments:

- in the general conduct of this experiment, it can be noticed that the energy and protein level of the used feed concentrate mixtures show the use of an intensity of rearing broilers without establishing feed force;

- in the starter phase, it is provided a 3000 kcal ME/kg and 22% CP using 35% corn, 10% wheat, 25% soybean meal, and 5% sunflower oil;
- during the rearing phase, there was used only one concentrate mixture structure, respectively $CM_{rearing}$ which provided 2990 kcal ME/kg and 20.03% CP

that can contribute to the continuity of intensity of rearing during the start phase, following the economic efficiency of such CM;
- during the finishing phase, we wanted to use reduction of protein compounds in the concentrate mixtures and increase metabolizable energy by adding high amounts of corn in CM structure.

Statistical processing of the results was performed by using SPSS 19 IBM program. Bio productive indices were set when the CM structures changed, respectively at the age of 2, 3, 7 weeks, and at the end of experiment (at the age of 10 weeks).

RESULTS AND DISCUSSION

The data regarding body weight (*Table 1*) registered during the growing phase of broilers in V_1 show that it significantly increased, from about 38 g at the age of 1 day to 1428.60 g, and the broilers in V_2 show a body weight increase from about 33 g to 1312.2 g during the 10 experimental weeks.

Table 1. Body weight of chickens belonging to different experimental groups

Item	Age (weeks)	Experimental variants		Difference		Student test
		V_1	V_2	absolute	relative %	
Body weight (g)	2	282.70±7.44	277.70±7.31	5.00	1.77	0.524is
	3	438.40±11.40	444.75±11.6	-6.35	1.45	0.260is
	7	1053.30±26.60	959.50±22.00	93.80	8.91	0.011*
	10	1428.60±38.40	1313.20±36.80	115.40	8.08	0.030*

The data in *Table 1* show that the body weight registered close values in both experimental variants in the first two rearing weeks. During that time the differences between V_1 and V_2 being very small. It was 2.95 g in the first week and 2.05 g in the second week, because CM was administered with the same nutritional features.

In the third week, it is noticed a slight difference between the average body mass of the broilers in the two experimental variants. It was about 1.45% in favour of the experimental variants 2, a group fed with $CM_{starter}$ structure.

Broilers in variant V_1 tri-phase fed began to significantly differ from broilers in V_2 from the fifth experimental week, and that difference maintained until the end of the rearing phase ($p<0.05$).

The result of statistical analyses of body weight differences registered between the two experimental variants during the rearing period are graphically represented in *Figure 1.*

It can be concluded from the analysis of the data on the evolution of body weight recorded during the 10 weeks of growth that the structure and features of concentrates mixtures administered to broilers during that period influenced their evolution. Thus resulted in a significantly higher growth of broilers in the variant fed with three CM structures (V_1) compared with chickens fed with two CM structures (V_2) even if the start period was a week longer.

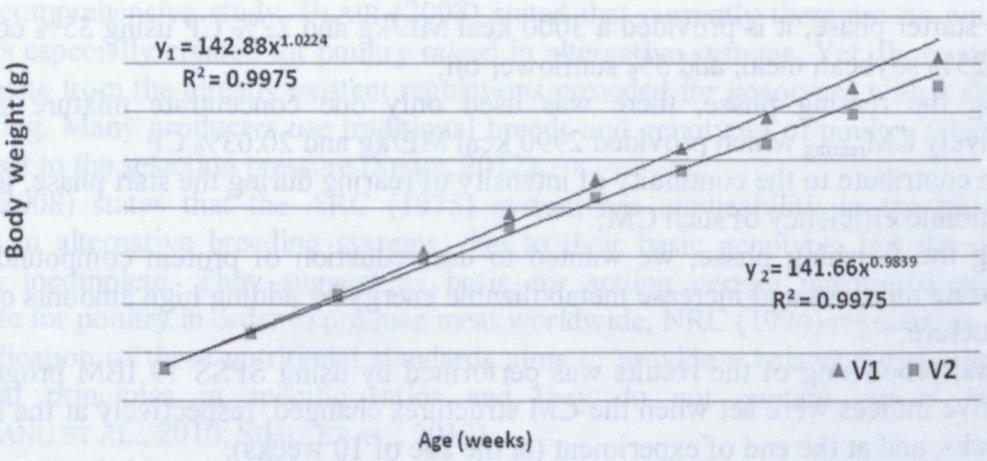

Figure 1. Evolution of body weight of broilers in the two experimental variant

Table 2. Concentrated mixtures intake, feed conversion factor and ME and CP intake in broilers belonging to all experimental variants

Item	Age (weeks)	Experimental variants		Differences	
		V_1	V_2	absolute	relative %
Intake CM (g/head)	2	511.00	516.00	-5.00	0.98
	3	833.00	846.00	-13.00	1.56
	7	2345.00	2296.00	49.00	2.09
	10	3696.00	3586.00	110.00	2.97
FCR (kg CM/kg weight gain)	2	2.09	2.15	-0.06	3.08
	3	2.08	2.08	0.00	0.00
	7	2.31	2.49	-0.18	7.88
	10	2.66	2.81	-0.15	5.80
Intake ME (kcal)	2	118.40	119.25	-0.85	0.71
	3	137.54	141.43	-3.89	2.83
	7	176.41	173.57	2.84	1.61
	10	204.00	195.00	9.00	4.41
Intake CP (g)	2	8.82	8.88	-0.06	0.68
	3	9.21	9.44	-0.23	2.50
	7	11.82	11.59	0.23	1.94
	10	13.12	12.55	0.57	4.34

From the analysis of the data presented in *Table 2*, it can be observed that the intake of concentrates mixture was close in both experimental variants, so at the end of the analyzed period, V_1 recorded a total of 3696 g of CM consumption, about 3% more than in V_2, which recorded a total consumption of 3586 g of CM.

Regarding this indicator, one can say that the administration periods and the nutritional characteristics of concentrate mixtures used did not significantly affect feed intake in broilers belonging to the two groups of the experiment.

According to these data, it is clear that the barred Plymouth Rock avian youth performed feed conversion ratios between 2.66 and 2.81 which are suitable for this rearing system. A percentage difference of 5.80% between V_1 and V_2 may recommend keeping the CM nutritional levels tested in the experiment, which allows maintaining the productive performance of this breed.

Regarding the metabolizable energy intake of broilers in the two experimental variants, according to the data in *Table 2* we can see that the differences between V_1 and V_2 are only

4.41%. Regarding the crude protein intake, the differences between the two versions are 4.34%.

Both in ME and in CP intake the differences identified during the 10 weeks are statistically insignificant ($p > 0.05$).

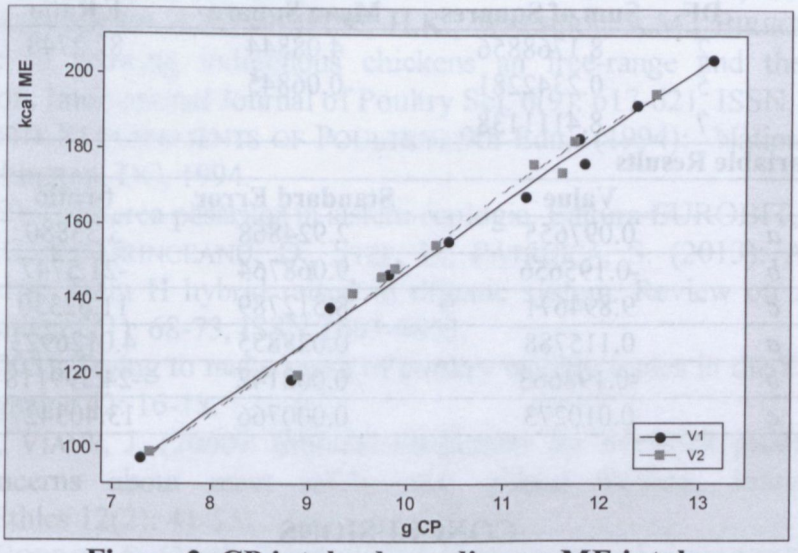

Figure 2. CP intake depending on ME intake

The graphical representation of the consumption of crude protein depending of the metabolizable energy intake shows that the recorded values were very close in both experimental variants (*Figure 2.)*.

The analyses of feeding cost established throughout the growth by acquisition price of CM feed and growth phases (*Table 3*).

As expected, according to the information in *Table 3* the highest feed costs are the broilers of V_1 with 1.53 euro / head, but in the case of relative costs per kg live weight, it can be observed that the broilers of V_1 such costs are only 1.87% higher than V_2 which allows the recommendation of a tri-phase feeding with nutritional values of CM and economy aspects as well.

Table 3. Fodder feeding costs of broilers in the experimental variants

Item	Experimental variants		Differences	
	V_1	V_2	absolute	relative %
Feeding cost/head (EUR)	1.53	1.37	0.16	10.45
Feeding cost/kg body weight (EUR)	1.07	1.05	0.02	1.87

Starting from the idea that the higher costs used for poultry rearing are registered for fodder feeding, using Data fit, an informational software, it was obtained a mathematic pattern that can predict the fodder feeding costs depending on CP intake.

The pattern: $y = a/(1+bx+cx^2)$ is shown in *Table 4*.

The correlation coefficients between the expenses incurred by fodder feeding (y) and crude protein intake (x) on *Table 4* show that there exists a strong positive correlation, as follows: 0.958 (in V_1) and 0.980 (in V_2).

This mathematical equation can assess the fodder feeding costs for Plymouth Rock chickens reared in free range system under similar conditions in which this experiment is conducted, based on the determination of crude protein intake.

Table 4. The assessment of fodder feeding costs depending on CP (g) intake

Model Definition: $y = a/(1+bx+cx^2)$,
where: y=CF, x=intake CP(g)

Variance Analysis					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**	**Probe (F)**
Regression	2	8.1768856	4.08844	87.2748	**0.0001**
Error	5	0.2342281	0.06845		
Total	7	8.4111138			

Regression Variable Results					
Variable		**Value**	**Standard Error**	**t-ratio**	**Probe (t)**
V_1	*a*	0.097655	2.924868	3.33880	**0.020**
$R^2=0.972$	*b*	-0.195656	9.068764	-21.5747	**0.000**
$r=0.958$	*c*	9.894671	8.512789	11.62330	**0.000**
V_2	*a*	0.115788	0.028855	4.0126923	**0.010**
$R^2=0.976$	*b*	-0.198663	0.008142	-24.399118	**0.000**
$r=0.980$	*c*	0.010273	0.000766	13.403427	**0.000**

CONCLUSIONS

In family farms of broiler rearing, including of the broilers belonging to mixed breeds (Plymouth Rock), there is an intention to simplify the feeding technology by reducing the number of structures of concentrates mixtures adapted to different rearing phases of avian youth. From this point of view, it has been analyzed the production and economic indicators performed in two experimental variants where the broilers were fed in tri-phase and bi-phase feeding system and with the nutritional AC features provided in the organization of the experiment.

Experimental data obtained allowed the following conclusions:

-broilers in V_1 tri-phase fed with CM $_{starter}$, CM $_{rearing}$ and CM $_{finishing}$ obtain during the experimental period a feed intake comparable with that of the broilers in V_2, an average body mass of 1428.60 g with a total increment higher with 8.3% and a better feed conversion (2.66 kg/kg) due to higher costs per kg body weight of livestock by 1.87% comparing with V_2;

-broilers in V_2 CM starter fed and CM rearing-finishing fed compared with V_1 registered an insignificant less feed intake (3586 g CM/broiler) a significantly less ($p<0.05$) average body mass of 1313.20 g and a weaker feed conversion of 2.81kg/kg (-5.6%), but by 1.87% smaller feeding costs per kg livestock than V_1.

- correlation coefficients between the costs registered with fodder feeding (y) and crude protein intake (x) show that there is a strongly positive correlation between them, thus: 0.958 (in V_1) and 0.980 (in V_2).

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